VITAMINS AND HORMONES

VOLUME V

VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

Edited by

ROBERT S. HARRIS *and* KENNETH V. THIMANN

Professor of Biochemistry of Nutrition
Massachusetts Institute of Technology, Cambridge, Mass.

Professor of Plant Physiology,
Harvard University,
Cambridge, Mass.

VOLUME V

1947
ACADEMIC PRESS INC., PUBLISHERS
NEW YORK

Copyright 1947, by
ACADEMIC PRESS INC.
125 EAST 23RD STREET
NEW YORK 10, N. Y.

PRINTED IN THE UNITED STATES OF AMERICA

CONTRIBUTORS TO VOLUME V

H. R. Bird, *Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Maryland*

Anne C. Carter, *Department of Medicine, Cornell University Medical College and the New York Hospital, New York, New York*

Harry A. Charipper, *Department of Biology, Washington Square College of Arts and Science, New York University, New York, New York*

Eugene J. Cohen, *Department of Medicine, Cornell University Medical College and the New York Hospital, New York, New York*

William J. Darby, *Department of Medicine and Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee*

C. W. Emmens, *National Institute for Medical Research, Hampstead, London, England*

Herbert M. Evans, *Institute of Experimental Biology, University of California, Berkeley, California*

Albert S. Gordon, *Department of Biology, Washington Square College of Arts and Science, New York University, New York, New York*

Carl G. Heller, *Departments of Physiology and Medicine, University of Oregon Medical School, Portland, Oregon*

Choh Hao Li, *Institute of Experimental Biology, University of California, Berkeley, California*

William O. Maddock, *Departments of Physiology and Medicine, University of Oregon Medical School, Portland, Oregon*

Daniel Melnick, *Chief of the Food Development Division, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois*

Nicholas A. Milas, *Massachusetts Institute of Technology, Cambridge, Massachusetts*

Harold P. Morris, *National Cancer Institute, National Institute of Health, Bethesda, Maryland*

Alexander von Muralt, *Halleranium, Berne, Switzerland*

Bernard L. Oser, *Food Research Laboratories, Inc., Long Island City, New York*

A. S. Parkes, *National Institute for Medical Research, Hampstead, London, England*

Ephraim Shorr, *Department of Medicine, Cornell University Medical College and the New York Hospital, New York, New York*

Editors' Preface

Articles concerned with the chemical, biological, and clinical properties of vitamins and hormones are appearing in ever-increasing numbers in the scientific journals of the world. In fact, it is now humanly impossible for one to keep abreast with all the advances in knowledge in these fields by reading the original articles. Scientists and clinicians must rely upon critical reviews to keep them informed of advances in fields related to their special interests.

Previous volumes of *Vitamins and Hormones* have been very favorably received. The Editors interpret this as evidence that this publication is fulfilling a need for critical reviews of the field. Suggestions and criticisms will always be welcome. An index to the contents of the five volumes now published is included at the end of this volume for reference.

Volume V was written and published during a period of post-war turbulence. The Editors wish to express their gratitude to the authors whose concentration and devotion under difficult circumstances have led to the publication of the excellent reviews in this volume.

While this preface is not, in general, the place for comments on the literature, the Editors would like to mention the following publication, of which notice has recently been received. "Register der Welt Literatur über Vitamine und der von ihnen beeinfluszten Gebiete" by Dr. M. Stechow, with a preface by Dr. A. Scheunert, is published by Helingsche Verlagsanstalt, Leipzig. The first volume of this massive tabulation of the world's vitamin literature covers the period 1890–1929, and the second volume has been promised for later years.

KENNETH V. THIMANN
ROBERT S. HARRIS

October, 1947

CONTENTS

Page

Contributors to Volume V v
Editors' Preface vii

The Synthesis of Vitamin A and Related Products

By Nicholas A. Milas, *Massachusetts Institute of Technology, Cambridge, Massachusetts*

I. Introduction 1
II. Synthesis of Biologically Active Homologues of Vitamin A Ethers . . . 4
III. Synthesis of Vitamin A Ethers 9
IV. Synthesis of Vitamin A Esters 22
V. Dimethylaminovitamin A and Its Conversion to Vitamin A 25
VI. Synthesis of "Vitamin A Acid" 27
VII. Stereochemistry of Vitamin A 30
References 36

Physiological Availability of the Vitamins

By Daniel Melnick and Bernard L. Oser, *Food Development Division, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois, and Food Research Laboratories, Inc., Long Island City, New York*

I. Introduction 39
II. Limitations in the Non-Biological Methods for Determining the Vitamins 41
    1. Physical Methods 41
    2. Chemical Methods 42
    3. Microbiological Methods 44
III. Measurement of Physiological Availability 47
    1. Animal Tests 48
    2. Human Assay Procedures 49
IV. Factors Influencing the Physiological Availability of the Vitamins . . . 57
    1. Fat-Soluble Vitamins 57
        a. Vitamin A 58
        b. Vitamin D 60
        c. Vitamin E 60
        d. Vitamin K 61
    2. Water-Soluble Vitamins 61
        a. Ascorbic Acid 62
        b. Thiamine 64
        c. Riboflavin 68
        d. Niacin 69
        e. Panthothenic Acid 72
        f. Vitamin B_6 72
        g. Biotin 73

Page

h. *p*-Aminobenzoic Acid 75
i. Folic Acid 75
j. Inositol 77
k. Choline 78
V. Unsolved Problems of Physiological Availability in Human Nutrition . . 79
1. Conditioned Malnutrition 79
2. Biosynthesis of Vitamins 80
3. Vitamin-Vitamin and Vitamin-Amino Acid Interrelationships 81
4. Antivitamins 82
5. Availability of the Water-Insoluble Forms of the Water-Soluble Vitamins 82
6. Availability of the Niacin Derivative in Cereal Products 83
7. Influence of Treating Foods with Various Salts During Processing . . 84
VI. Concluding Remarks 84
References 85

Thiamine and Peripheral Neurophysiology

BY ALEXANDER VON MURALT, *Halleranium, Berne, Switzerland*

I. Introduction 93
II. Liberation of Thiamine in Nervous Excitation 93
III. Thiamine Content of Peripheral Nerves 105
IV. Liberation of Thiamine on Vagus Stimulation in the Heart 110
V. Discussion and Summary 114
References 116

The Physiological Effects of the Pteroylglutamates in Man—with Particular Reference to Pteroylglutamic Acid (PGA)

BY WILLIAM J. DARBY, *Department of Medicine and Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee*

I. Introduction 119
1. Nomenclature 120
2. Historical 122
II. Sprue 124
1. Clinical Description 124
2. Effects of Pteroylglutamates 125
a. Experimental Background 125
b. Clinical and Hematological Effects 126
c. Effects on the Gastrointestinal Tract 131
3. Estimated Daily Requirements of Vitamin M 136
III. Nutritional Macrocytic Anemia 139
IV. Macrocytic Anemia of Pregnancy (Pernicious Anemia of Pregnancy) . . . 140
V. Pernicious Anemia 141
1. Clinical Description 141
2. Effects of Pteroylglutamates 142
a. General Clinical 142
b. Reticulocyte Response 144
c. Erythrocyte Response 147

Page
d. Other Effects 149
3. Evidence Relative to the Nature of a Metabolic Defect 150
VI. Pteroylglutamates in the Treatment of Diseases of Infancy and Childhood 152
VII. Pteroylglutamates in the Treatment of Chronic Diarrheas 154
VIII. Thymine 154
IX. A Resumé of the Nutritional Physiology of Vitamin M in Man 155
References 157

The Vitamin Requirements of Chicks

By H. R. Bird, *Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Maryland*

I. Introduction 163
II. Vitamin A 165
III. Vitamin D 165
IV. Thiamine 166
V. Riboflavin 166
VI. Pantothenic Acid 166
VII. Niacin, Pyridoxin, Biotin 167
VIII. Vitamin K 167
IX. Choline 167
X. Other Factors 168
XI. Requirements of Other Avian Species 169
XII. Vitamin Content of Purified Diets Currently Used 170
XIII. Summary 171
References 171

Vitamin Requirements of the Mouse

By Harold P. Morris, *National Cancer Institute, National Institute of Health, Bethesda, Maryland*

I. Introduction 175
II. Thiamine 176
III. Riboflavin 180
IV. Pantothenic Acid 182
V. Pyridoxine 185
VI. Biotin and Folic Acid 188
VII. Niacin and Choline 189
VIII. Inositol 189
IX. Ascorbic Acid 189
X. Vitamins A and D 190
XI. Vitamin E 190
XII. Mouse Growth Factor in Proteins 191
XIII. Vitamin Analogues 191
1. Pantoyltaurine 191
2. Riboflavin Analogue 192
3. Niacin Analogues 192
4. Thiamine Analogues 192
5. α-Tocopherol and Vitamin K Analogues 193

Page
XIV. Summary 193
References 194

The Properties of the Growth and Adrenocorticotropic Hormones

By Choh Hao Li and Herbert M. Evans, *Institute of Experimental Biology, University of California, Berkeley, California*

I. Introduction 198
II. Growth Hormone 199
  1. Methods of Assay 199
    a. Body Growth of Normal Female Rats 199
    b. Body Growth of Hypophysectomized Female Rats 200
    c. Tibia of Hypophysectomized Rats 200
  2. Method of Isolation 201
    a. Historical 201
    b. The Procedure 202
    c. Homogeneity Studies 203
  3. Physical and Chemical Properties 206
    a. Isoelectric Point and Molecular Weight 206
    b. Analytical Data 207
    c. Diffusion and Viscosity 207
    d. Stability 207
  4. Biological Properties 208
    a. Body Growth 208
    b. Nitrogen Retention 211
    c. Stimulation of Cartilage Proliferation 213
    d. Diabetogenicity 213
III. Adrenocorticotropic Hormone 215
  1. Methods of Assay 215
    a. Historical 215
    b. The Repair Test 216
    c. The Maintenance Test 216
  2. Methods of Isolation 216
    a. Swine Hormone 218
    b. Sheep Hormone 218
  3. Physical and Chemical Properties 220
    a. Isoelectric Point 220
    b. Molecular Kinetic Data 220
    c. Some Analytic Data 220
    d. Solubility 220
    e. Stability 220
    f. Effect of Various Reagents 221
  4. Biological Properties 221
    a. Inhibition of Growth 221
    b. Effect on the Gonads of Male Rats 222
    c. Effect on Carbohydrate Metabolism 224
    d. Effect on Lymphoid Tissues and Serum Proteins 225
    e. Effect on Cholesterol and Ascorbic Acid Content of the Adrenal . . 226
References 227

Page

Effect of Exogenous Estrogens on the Male Mammal

BY C. W. EMMENS AND A. S. PARKES, *National Institute for Medical Research, Hampstead, London, England*

I. Introduction 233
II. Chemical Structure of Estrogens 234
III. Endogenous Estrogens in the Male 234
IV. The General Effects of Estrogens on Adult Males 236
  1. The Skeleton 236
  2. External Genitalia and the Skin 238
  3. Internal Genitalia 239
  4. The Ejaculate 240
V. The General Effects of Estrogens on Immature Males 241
  1. Sex Differentiation 241
  2. Postnatal Development 242
VI. Effects on the Pituitary Gland 248
  1. Morphological and Histological Changes 248
  2. Changes in the Content of Gonadotropic Hormones 250
VII. Direct Effects on the Accessory Reproductive Organs 251
  1. Seminal Vesicles and Prostate 251
  2. Mammary Gland 259
VIII. Effects on the Adrenal Glands 262
IX. Interaction of Estrogenic and Androgenic Substances 263
  1. Protection of Males from the Effects of Estrogens 263
  2. Synergistic Actions of Estrogens and Androgens 266
References 267

The Biology of Antithyroid Agents

BY HARRY A. CHARIPPER AND ALBERT S. GORDON, *Department of Biology, Washington Square College of Arts and Science, New York University, New York, New York*

I. Introduction 274
II. Historical Survey 275
III. Effects on the Gross and Microscopic Structure of the Thyroid and Pituitary Glands 277
  1. Mammals 277
    a. Thyroid Changes 277
    b. Reversibility of Effect 279
    c. Pituitary Changes 279
  2. Birds 280
  3. Reptiles 281
  4. Amphibians 281
  5. Fishes 282
  6. Invertebrates 283
  7. During Ontogeny 283
IV. Effects on Body Growth and General Metabolism 283
  1. Mammals 283
  2. Birds 285
  3. Amphibians 286
  4. Fishes 286

Page

V. Mechanism of Action 287
  1. Effects on Iodine Metabolism 287
    a. *In Vivo* Experiments 287
    b. *In Vitro* Experiments 289
  2. Effects on Enzyme Systems 290
    a. Peroxidase 290
    b. Cytochrome Oxidase 291
    c. Succinoxidase 291
    d. Xanthine Oxidase 291
    e. Phosphatase 291
    f. Tyrosinase 292
    g. Proteolytic Activity 292
    h. Oxidation-Reduction Potentials and Enzyme Activity 292
  3. Effects on the Pituitary Thyrotropin Mechanism 293
  4. Direct Effects on the Thyroid 295
VI. Effects on Organs Other than the Thyroid and Pituitary 295
  1. Adrenal Gland 295
  2. Reproductive Organs and Accessories 296
  3. Liver and Kidney 297
  4. Peripheral Blood and Bone Marrow 297
  5. Other Organs 298
VII. Factors Influencing Activity of Antithyroid Agents 299
  1. Age 299
  2. Sex 299
  3. Diet 300
  4. Temperature 300
  5. Methods of Administration 300
  6. Species Differences 301
VIII. Metabolism of Antithyroid Agents 301
IX. Chemical Nature of Antithyroid Agents 303
X. Clinical Applications 305
XI. Conclusion 307
  References 307
  Addendum 315

The Use of Androgens in Women

By Anne C. Carter, Eugene J. Cohen and Ephraim Shorr, *Department of Medicine, Cornell University Medical College and the New York Hospital, New York, New York*

I. Introduction 318
II. Relative Potencies and Modes of Administration of Androgens 319
III. Biological Effects of Androgens in Women 325
  1. Anterior Pituitary 326
  2. Ovary 327
  3. Uterus 329
    a. Endometrium 329
    b. Myometrium 330
    c. Fallopian Tubes 331

Page

4. Vaginal Epithelium and Secretions 331
5. Menstruation 333
6. Breast. 334
7. Virilizing Effects 334
    a. Acne. 334
    b. Hypertrichosis. 335
    c. Vulva and Clitoris 335
    d. Voice 336
8. Urinary Tract 336
    a. Kidney. 336
    b. Bladder. 337
9. Urinary 17-Ketosteroids. 337
10. Metabolic Effects. 338
11. Vascular Effects 338
12. PsychobiologicalEffects 339
IV. The Clinical Uses of Androgens 339
1. Uterine 339
    a. Functional Bleeding 339
    b. In Association with Myoma. 345
2. Pelvic Disorders 347
    a. Endometriosis. 349
    b. Pelvic Inflammatory Disease 350
    c. Endocervicitis. 350
    d. Malignancy of the Genital Tract. 350
    e. Functional Urinary Disturbances. 350
3. Dysmenorrhea 351
4. Premenstrual Tension. 356
5. The Menopausal Syndrome 358
6. Libido 361
7. Functional and Pathological Disorders of the Breast 364
    a. Breast Pain and Benign Lesions 364
    b. The Painful Postpartum Breast and Lactation. 369
    c. Breast Carcinoma: Primary and Metastatic. 371
8. Disorders Related to Pregnancy 373
    a. Nausea and Vomiting. 373
    b. After-Pains 374
9. Metabolic and Endocrine Disorders 374
    a. Cushing's Syndrome 374
    b. Postmenopausal Osteoporosis 376
    c. Simmonds' Disease. 376
    d. Acromegaly. 376
    e. Pituitary Dwarfism. 377
    f. Addison's Disease 377
    g. Graves' Disease 378
    h. Muscular Dystrophy. 378
    i. Debilated States. 378
10. Miscellaneous 378
    a. Diseases Characterized by Menstrual Exacerbations 378
    b. Diseases with a High Incidence in Women. 378

Page

c. Disorders of the Skin . . . 381
d. Psychiatric Disorders . . . 381
V. Conclusions . . . 382
References . . . 383

The Clinical Uses of Testosterone in the Male

By Carl G. Heller and William O. Maddock, *Departments of Physiology and Medicine, University of Oregon Medical School, Portland, Oregon*

I. Introduction . . . 394
II. Hypogonadism . . . 394
1. Evaluation of Laboratory Determinations that Might Aid in Distinguishing Hypogonadism from Normal . . . 395
a. Urinary Androgen Excretion . . . 395
b. Urinary Estrogenic Activity . . . 396
c. Urinary 17-Ketosteroids . . . 396
d. Urinary Gonadotropins . . . 397
e. Testicular Biopsies . . . 398
2. Functional Pathology of Hypogonadism . . . 398
a. Normal Mechanisms . . . 398
b. Functional Pathology . . . 400
3. Clinical Features . . . 400
a. Secondary or Hypogonadotropic Hypogonadism . . . 400
b. Primary or Hypergonadotropic Hypogonadism . . . 404
4. Details of the Hypergonadotropic Syndromes . . . 406
a. Hypergonadotropic Eunuchoidism . . . 406
b. Adult Hypergonadotropic Hypogonadism . . . 408
c. Puberal Seminiferous Tubule Failure . . . 410
d. Adult Seminiferous Tubule Failure . . . 411
III. Testosterone Administration . . . 411
1. Inunction . . . 412
2. Oral . . . 412
3. Sublingual . . . 412
4. Intramuscular or Subcutaneous Injection . . . 413
5. Pellet Implantation . . . 413
IV. Changes Effected by Androgen Administration . . . 414
V. Effect of Exogenous Testosterone on Spermatogenesis . . . 415
VI. Use of Testosterone in the Prepuberal Child . . . 417
VII. Cryptorchidism . . . 417
VIII. Impotence . . . 418
IX. Homosexuality . . . 419
X. Angina Pectoris . . . 422
XI. Benign Prostatic Hypertrophy . . . 425
XII. Conclusions . . . 427
XIII. Summary . . . 427
References . . . 428

Author Index . . . 433
Subject Index . . . 460
Cumulative Index of Vols. I-V . . . 475

The Synthesis of Vitamin A and Related Products

By NICHOLAS A. MILAS

CONTENTS

Page

I. Introduction 1
II. Synthesis of Biologically Active Homologues of Vitamin A Ethers 4
III. Synthesis of Vitamin A Ethers 9
IV. Synthesis of Vitamin A Esters 22
V. Dimethylaminovitamin A and Its Conversion to Vitamin A 25
VI. Synthesis of "Vitamin A Acid" 27
VII. Stereochemistry of Vitamin A 30
References 36

I. Introduction

Since the elucidation of the structure of vitamin A by Karrer, Morf and Schöpp (1931), Karrer and Morf (1933), Heilbron, Heslop *et al.* (1932) and Heilbron, Morton *et al.* (1932) and by others, several synthetic approaches to the vitamin itself or its derivatives have been published. These have been ably reviewed elsewhere by Bogert (1938), by Sobotka and Bloch (1944) and by Heilbron, Jones and Bacharach (1944).

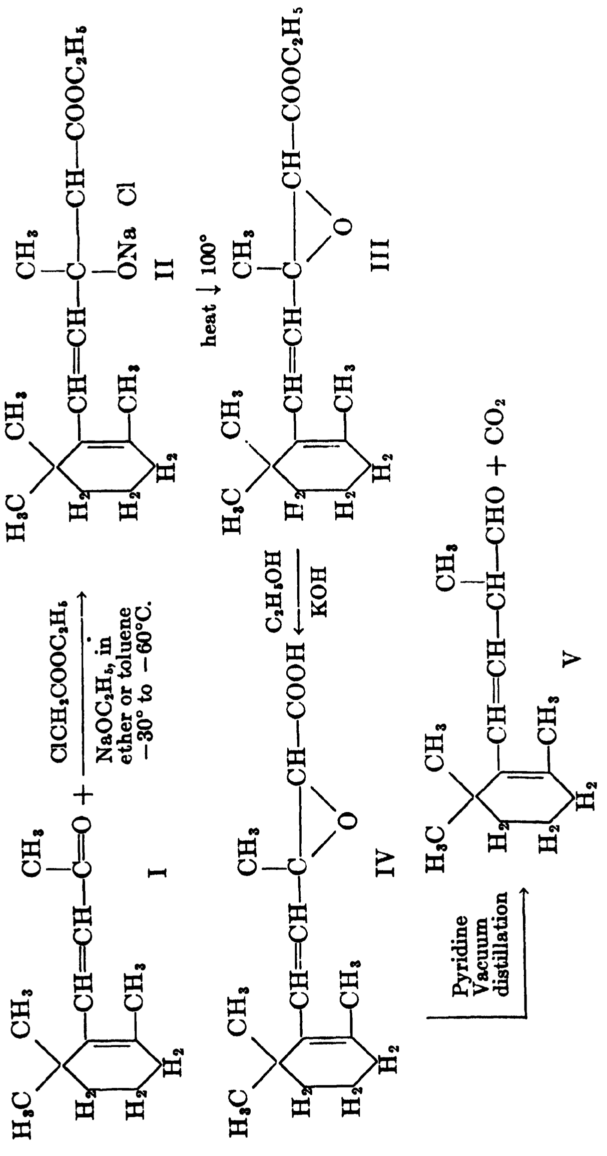
The first claim to a synthesis of vitamin A was made by Fuson and Christ (1936). By allowing β-cyclocitral to condense with β-methylcrotonaldehyde and reducing the resulting product with aluminum isopropoxide, they obtained an alcohol which was assumed to be vitamin A. Spectroscopic evidence was the only proof given to support this claim, which was later disputed by Heilbron and Jones (1936) and by Kuhn and Morris (1937) on the grounds that β-cyclocitral fails to condense with β-methylcrotonaldehyde and the product of Fuson and Christ was merely an open chain polyene aldehyde formed by the autocondensation of β-methylcrotonaldehyde.

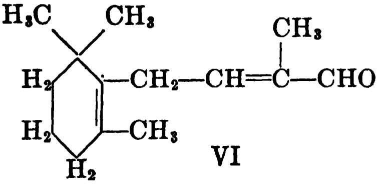
Following along similar lines, Kuhn and Morris (1937; 1940; 1941a,b) published a synthesis of vitamin A in which β-ionylideneacetaldehyde, instead of β-cyclocitral, was condensed with β-methylcrotonaldehyde in the presence of piperidine acetate as a catalyst, and the resulting product, without purification, reduced with aluminum isopropoxide. The mixture obtained was then chromatographed on alumina from which was eluted a specimen having an absorption maximum with antimony trichloride in chloroform of 606 mμ and a biological activity of about 7.5% of the purest vitamin A sample then known. No other analytical data or yields were given by the authors. In view of the importance of vitamin

A during the war, considerable time was spent by several investigators (Sobotka and Bloch, 1944; Heilbron, Jones and Bacharach, 1944; Karrer and Rüegger, 1940; Krauze and Slobodin, 1940) in an effort to repeat this synthesis. In no case was a biologically active product obtained. This synthesis was also investigated in our Laboratory (Milas and Kovitz, 1947), but our failure to obtain β-ionylideneacetaldehyde forced us to abandon it early in 1940. β-Ionylideneacetaldehyde, the key intermediate in the Kuhn-Morris synthesis, has not yet been obtained in the pure state. The original claim of Davies, Heilbron *et al.* (1935) that this aldehyde can be prepared by heating *in vacuo* a mixture of barium β-ionylideneacetate and barium formate has been disputed by Sobotka, Bloch and Glick (1943) who obtained α-ionone and no aldehyde, while, in a more recent study of the same reaction, Karrer and Rüegger (1944) reported the formation of β-ionone. Instead of the barium salts, we heated, under greatly reduced pressure, the corresponding thorium salts and obtained no product possessing aldehydic properties (Milas and Sakal, 1947). A 64% yield of β-ionylideneacetaldehyde has been reported by Krauze and Slobodin (1940) from a reaction of β-ionone with bromoacetal and magnesium. This synthesis was also repeated in this laboratory but no β-ionylideneacetaldehyde was obtained, instead β-ionone was recovered unchanged (Milas and Edgerton, 1947).

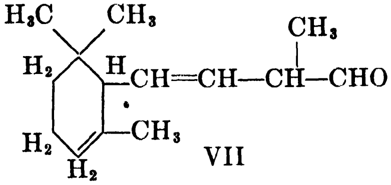
Following a somewhat different scheme (Milas and McAlevy, 1935), Kipping and Wild (1939) published a synthesis of vitamin A methyl ether in which β-ionone was condensed with 1-bromo-4-methyl-6-methoxyhexadiene-2,4 using lithium as the condensing agent and the resulting product dehydrated. Since no experimental details, analyses or biological results were given, it is difficult to evaluate this synthesis.

Early in 1940, the present author (Milas, 1945a–o; Milas, 1946) with several of his associates launched an exhaustive study of several routes, other than those mentioned above, for the synthesis of vitamin A, its derivatives, and its homologues. The key intermediate of several of these syntheses, which ultimately led to the preparation of biologically active vitamin A products, was prepared by the application of the Darzens' synthesis to β-ionone (Milas, 1945a–o; Milas, 1946; Ishikawa and Matsuura, 1937; Heilbron, Johnson *et al.* 1942; Cymerman *et al.* 1946; Milas, Lee, Sakal *et al.* 1947). When β-ionone was condensed with ethyl chloroacetate at low temperatures (−30° to −60°C.) in anhydrous ether or toluene, using alcohol-free sodium ethoxide or methoxide as condensing agents, the glycidic ester (III) was produced, which, upon hydrolysis, gave the glycidic acid (IV). This acid has been decarboxylated by various methods, but the best results were obtained when its pyridine salt was distilled under reduced pressure.


I
ClCH₂COOC₂H₅
NaOC₂H₅, in ether or toluene −30° to −60°C.
II
heat 100°
III
C₂H₅OH
KOH
IV
Pyridine Vacuum distillation
V
+ CO₂



VI



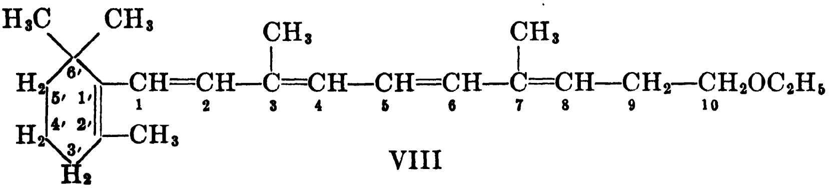
VII

The aldehyde formed by the decarboxylation of the glycidic acid (IV) was extensively investigated by Milas, Lee *et al.* (1947), who demonstrated that it probably has structure (V) rather than structure (VI) proposed by Heilbron, Johnson *et al.* (1942). Ozonization experiments, for example, led to the isolation of geronic acid rather than 3,3-dimethyloctanedione-2,7 which was expected to result from an aldehyde having structure (VI). The formation of small amounts of the isomeric aldehyde (VII) cannot be entirely avoided in spite of the high purity of the β-ionone used. This is not at all surprising in view of the recent work of Köster (1944) who reported that when pure β-ionone was treated with sodium alcoholate or with alcoholic alkali, it was partially converted into α-ionone. However, when pure α-ionone was treated with the same reagents, it was chiefly converted into β-ionone.

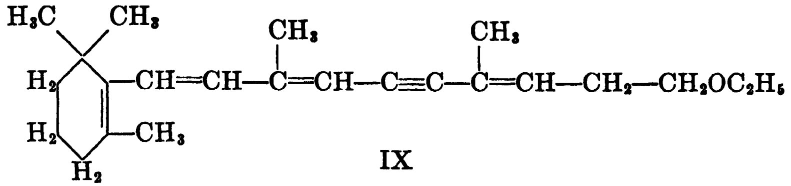
II. Synthesis of Biologically Active Homologues of Vitamin A Ethers

Two of the earliest biologically active products to be synthesized using the aldehyde (V) as the key intermediate, were [1-(2′,6′,6′-trimethyl-cyclohexen-1′-yl)-3,7-dimeth yldeca-1,3,5,7-tetraenyl]-10-ethyl ether (VIII) or simply homovitamin A ethyl ether, and [1-(2′,6′,6′-trimethylcyclohexen-1′-yl)3,7-dimethyldeca-1,3,7-trien-5-ynyl]-10-ethyl ether or simply 5-dehydrohomovitamin A ethyl ether (IX). These two products have been synthesized by a number of routes (Milas, 1945d; Milas, Lee *et al.* 1947).

In the first step of the synthesis of the 5-dehydrohomovitamin A ethyl ether, the aldehyde (V) was allowed to react in anhydrous ether



VIII



IX

with the Grignard of either 3-methyl-6-ethoxyhexa-3-en-yne-1 (X) or that of 3-methyl-6-ethoxyhexa-1-yn-3-ol (XI). In the first case the carbinol (XII) was formed, while in the second case the glycol (XIII) resulted. Both the carbinol and the glycol were dehydrated in toluene using catalytic amounts of *p*-toluenesulfonic acid to give *inter alia* 5-dehydrohomovitamin A ethyl ether. The crude sample exhibited two bands in the ultraviolet; a broad band at 316–320 mμ and another at 285–290 mμ. It was distilled once under high vacuum and the distillate tested biologically by Professor Robert S. Harris of the Nutritional Laboratories of this Institute. He reported that, when fed to vitamin A-deficient rats in doses of 96γ per day, it cured xerophthalmia and caused an average weight increase per rat of 32 g. for the 28-day test period (see Table III).

Further purification of the 5-dehydrohomovitamin A ethyl ether obtained from the dehydration of the carbinol (XII) was effected first by high vacuum distillation followed by partition between equal volumes of petroleum ether and 90% methanol, then by chromatographic adsorption of the petroleum ether-soluble portion on activated alumina. The unadsorbed portion (main product) was fractionated and the main fraction (light orange oil) boiling at 100–104°C. (10^{-5} mm.) collected and analyzed. It was found to have a single absorption band with a maximum at 321 mμ (Fig. 1, curve A) and to give a purplish-blue color with antimony trichloride in chloroform with absorption maxima at 622 and 580 mμ, respectively. Other analytical data such as hydrogenation and carbon and hydrogen analysis were in accord with formula (IX). The eluate showed two sets of bands very much like those of the original crude product.

The 5-dehydrohomovitamin A ethyl ether obtained from the dehydration of the glycol (XIII) was one of the earliest investigations of the authors and the product was purified in the same manner as in the above case, except that, instead of chromatographing the final product, it was fractionated in absolute methanol at temperatures between 0° and −78°C. following a method described elsewhere (Milas, 1939; Milas, Heggie and Mason, 1947). The product insoluble in methanol below −40°C. was recovered and examined spectroscopically. It was found to have a single band with a maximum at 321 mμ (Fig. 1, curve C) having a slightly lower extinction coefficient than the previous sample, showing that it was slightly less pure.

When a sample of 5-dehydrohomovitamin A ethyl ether having an $E^{1\%}_{1\,cm.}$ (321 mμ) of 1250 was partially hydrogenated in absolute ethano in the presence of 1% palladium deposited on calcium carbonate in a specially designed semimicro hydrogenation apparatus (Rivers and Milas, 1947), a product was obtained the absorption band of which was found at 320–321 mμ, $E^{1\%}_{1\,cm.}$ 1000 with a faint inflection at 290 mμ(?).

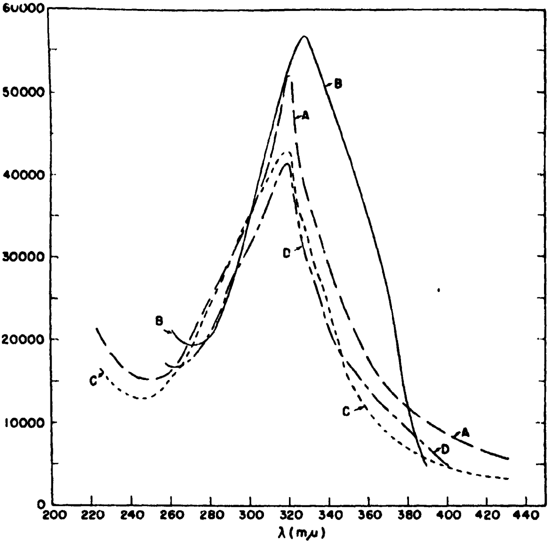
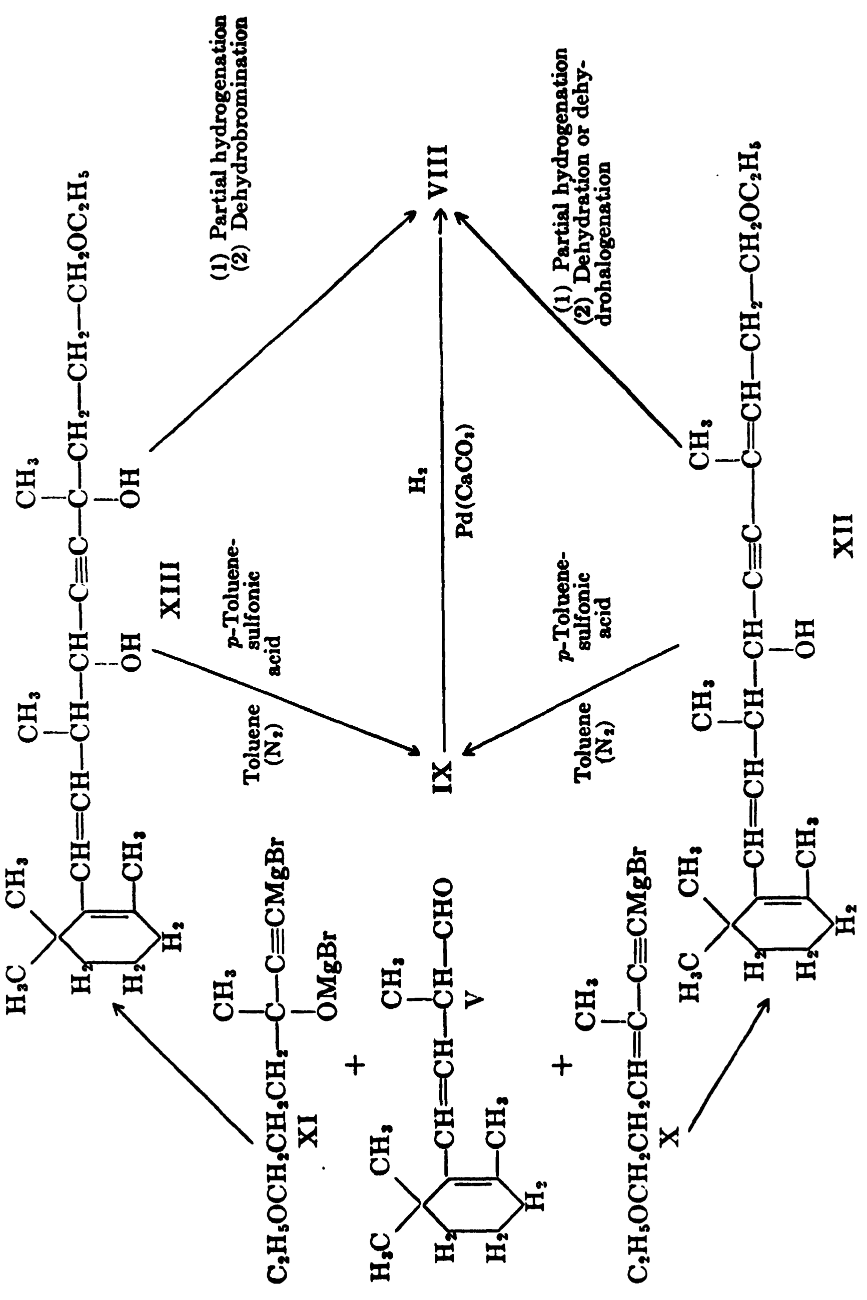


FIG. 1.—Absorption spectra in ethanol of: (A) 5-dehydrohomovitamin A ethyl ether from (XII); (B) homovitamin A ethyl ether *via* dehydrobromination (pyridine hydrobromide) of partially hydrogenated (XIII); (C) 5-dehydrohomovitamin A ethyl ether from (XIII); (D) homovitamin A ethyl ether *via* dehydration of partially hydrogenated (XII).

These results seem to indicate that the addition of hydrogen on the en-yne system of the 5-dehydrohomovitamin A ethyl ether had taken place mainly on the acetylene bond, since 1,4-addition would have produced an allene which should be spectroscopically equivalent to 5.5 double bonds (Kuhn and Wallenfels, 1938; Shantz, Cawley and Embree, 1943) and its absorption maximum should have been in the region of about 340 mμ. However, the possibility of a rearrangement of the allene into an en-yne with one less double bond should not be excluded. Such an en-yne should have a prominent absorption band in the region of 290 mμ. The fact that a faint inflection was found in this region indicates that some of the hydrogen had actually added 1,4 with a subsequent rearrange-


$C_2H_5OCH_2CH_2CH_2-C(CH_3)(OMgBr)-C{\equiv}CMgBr$
XI
+
V
+
$C_2H_5OCH_2CH_2CH{=}C(CH_3)-C{\equiv}CMgBr$
X
XIII
XII
(1) Partial hydrogenation
(2) Dehydrobromination
p-Toluene-sulfonic acid
Toluene (N₂)
H₂
Pd(CaCO₃)
IX
VIII
(1) Partial hydrogenation
(2) Dehydration or dehydrohalogenation
p-Toluene-sulfonic acid
Toluene (N₂)

ment of the allene formed. The problem of selective hydrogenation of symmetrically conjugated dieneyne systems will be discussed more fully in Section III.

Homovitamin A ethyl ether was also made by two other routes. When one mole equivalent of hydrogen was added to carbinol (XII) in the presence of 1% palladium on calcium carbonate, the corresponding tetraenol ethyl ether (XIV) was formed. Here too, the possibility of 1,4-addition to the en-yne system should not be entirely excluded, although the fact that the final product had a spectrum very similar to that of products obtained by other methods indicates that the addition was mainly on the acetylene bond. The tetraenol ethyl ether was dehydrated in toluene using catalytic amounts of *p*-toluene sulfonic acid, and the product obtained purified by distillation followed by partition between petroleum ether and 90% methanol, then by fractionation at low temperatures in absolute methanol. The final product was found to have a single band with a maximum at 321 mμ (Fig. 1, curve D).

A sample of the carbinol (XIV) was also dehydrochlorinated by treating it first with thionyl chloride in pyridine to convert it to the corresponding chloride which was treated with alcoholic potash to remove one mole of hydrogen chloride. The mixture obtained was partitioned between petroleum ether and 95% methanol and the petroleum ether portion, without further purification, was examined spectroscopically. It showed two bands: one at 282–290 mμ, $E_{1\,\text{cm.}}^{1\%}$ 406, and the other at 320–323 mμ, $E_{1\,\text{cm.}}^{1\%}$ 313. It also gave a deep blue color with antimony trichloride in chloroform.

When one mole equivalent of hydrogen was added to the glycol (XIII), in the presence of 1% palladium on calcium carbonate, the glycol (XV) was formed. The dehydrobromination of this glycol, using phosphorus tribromide in pyridine and subsequently treating the dibromide with alcoholic potash, was one of the earliest experiments of this investigation. A sample of the crude product was found biologically active when tested on vitamin A-deficient rats in doses of about 60 γ per day, but it was found highly unstable during the latter part of the feeding experiments.

More recently the glycol (XV) was dehydrobrominated in pyridine at 100°C. using pyridine hydrobromide, then treating the resulting product for a short time with alcoholic potash at 100°C. The crude product isolated from this reaction had a single broad band between 320 and 340 mμ. It also gave a deep blue color with antimony trichloride in chloroform. This product was further purified by partition between petroleum ether and 90% methanol followed by chromatographic adsorption of the petroleum ether portion on activated alumina, then by fractionation of the unadsorbed fraction at temperatures between 0° and −78°C. A yellow, highly viscous product was finally obtained which exhibited a prominent band with a maximum at 328 mμ and a faint indication at 367 mμ (Fig. 1, curve B). It has not been definitely established that this product is identical in every respect with that obtained by the direct dehydration of the carbinol (XIV).

It may be of interest to note that in all of the above cases and others to be discussed under Sections III and IV in which a triple bond is catalytically partially hydrogenated, the double bond formed may be a *cis*-double bond, although according to Zechmeister (1944), the 5-double bond in natural carotenoids and in vitamin A is so sterically hindered that it exists only in the *trans*-configuration. This point will be discussed more fully later.

A summary of the spectroscopic data of important products involved in the various syntheses which were discussed under this section is given in Table 1. It is highly unfortunate that at the time of the completion of this review no biological results were available on the purified final products.

TABLE I

Summary of Ultraviolet Absorption Spectra Data (in Alcohol)

Compound	λ max. (mμ)	ε mol.	log ε mol.
Glycidic ester (III)	286	22,040	4.34
Glycidic acid (IV)	286	34,080	4.53
Aldehyde (V)	232	20,518	4.31
Carbinol (XII)	233	21,500	4.33
Glycol (XIII)	220–223	4,470	3.65
5-Dehydrohomovitamin A ethyl ether (IX) from (XII) *via* dehydration	321	52,000	4.72
5-Dehydrohomovitamin A ethyl ether (IX) from (XIII) *via* dehydration	321	42,500	4.63
Homovitamin A ethyl ether (VIII) from (XIV) *via* dehydration	321	41,250	4.62
Homovitamin A ethyl ether from (XV) *via* dehydrobromination (pyridine hydrobromide)	328 367[1]	56,750 30,000	4.75 4.48

[1] Faint inflection.

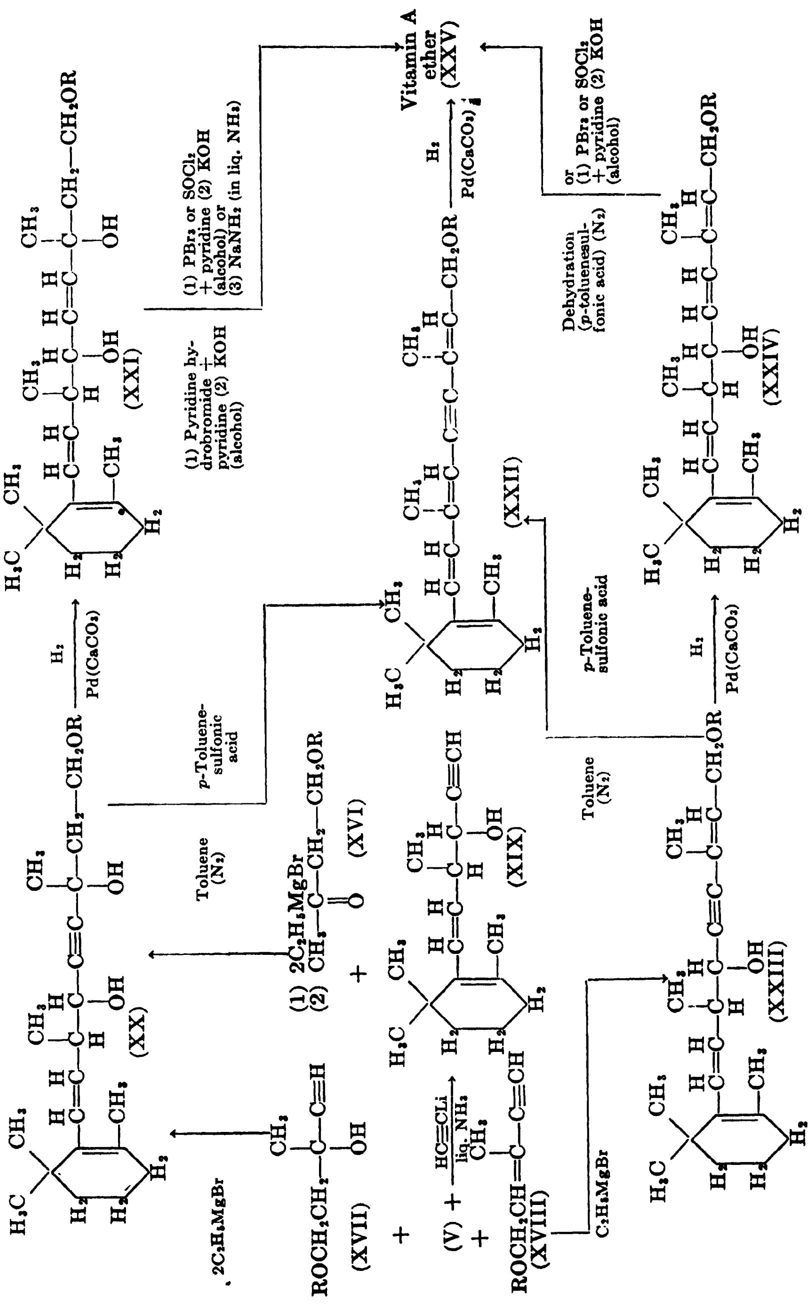
III. SYNTHESIS OF VITAMIN A ETHERS

Biologically active vitamin A ethers and their 5-dehydrovitamin A derivatives were first synthesized during the early stages of World War II by Milas and co-workers (Milas 1945b, 1945o; Milas, Sakal *et al.* 1947) employing somewhat analogous reactions to those described under

Section II. Only two ethers, the methyl and the ethyl, were synthesized in relatively pure form, while the isopropyl and *tert*-butyl were obtained in much less pure state.

In the synthesis of these ethers, 4-alkoxybutanone-2 (XVI), in which R represents methyl, ethyl, isopropyl or *tert*-butyl groups, was condensed with lithium acetylide in liquid ammonia to give the acetylene carbinol (XVII) which was dehydrated at 250°–280°C. in the presence of aluminum phosphate to give the vinyl acetylene (XVIII). In their earliest experiments, the authors condensed the aldehyde (V) with lithium acetylide in liquid ammonia to produce the acetylene carbinol (XIX). When the Grignard of (XIX) was allowed to react with 4-alkoxybutanone-2, the glycol (XX) was produced in 70–80% yields. This glycol has been obtained in two forms: a crystalline and a highly viscous liquid form. Since the double bond between carbon atoms 1 and 2 can exist only in *trans* form (Zechmeister, 1944), the difference between the crystalline and the liquid glycols may be one of *racemic* and *meso* forms. The two forms of the glycol (XX) were also synthesized in higher yields by condensing the Grignard of the carbinol (XVII) with the aldehyde (V). The partial hydrogenation of (XX) to give (XXI) was found to be selective when 1% palladium deposited on calcium carbonate was used as the catalyst.

Several methods were employed in the conversion of the glycol methyl ether (XXI) into the vitamin A methyl ether (XXV). Using phosphorus tribromide in the presence or absence of pyridine, the glycol was converted into the dibromide which was dehydrobrominated with alcoholic potash. The reaction was also studied with phosphorus trichloride, phosphorus triiodide and thionyl chloride. With thionyl chloride in pyridine, partial dehydrochlorination occurred as was indicated by the appearance in the spectrum of the dichloride of a broad band between 300 and 330 mμ. Of all the halogenating agents, phosphorus tribromide and thionyl chloride gave the best results. In all cases, however, the product obtained (a light yellow oil), after a single high vacuum distillation, was found to have two bands in the ultraviolet; one with a maximum at 325 mμ, and the other at 285–290 mμ. With antimony trichloride in chloroform, it gave a blue color which also showed two bands (Fig. 2, curves with broken lines); one with a maximum at 580 mμ, and another at 618–620 mμ. Repeated distillation from a shallow vessel at 10^{-5} mm. was detrimental to the chromogen responsible for the absorption band at 325 mμ. This is illustrated in Table II. A crude sample was first fractionated at low temperatures using methanol as the solvent, and the fraction having an $E^{1\%}_{1\,\mathrm{cm.}}$ (325 mμ) value of 1000 was distilled several times and examined spectroscopically.


2C₂H₅MgBr
ROCH₂CH₂—C(CH₃)(OH)—C≡H
(XVII)
+
(XX)
Toluene (N₂)
(1) 2C₂H₅MgBr
(2) CH₃—C(=O)—CH₂—CH₂OR
(XVI)
+
p-Toluene-sulfonic acid
H₂
Pd(CaCO₃)
(XXI)
(1) Pyridine hydrobromide + pyridine (2) KOH (alcohol)
(1) PBr₃ or SOCl₂ + pyridine (2) KOH (alcohol) or (3) NaNH₂ (in liq. NH₃)
(V) +
HC≡CLi
liq. NH₃
(XIX)
+
ROCH₂CH=C(CH₃)—C≡CH
(XVIII)
C₂H₅MgBr
(XXII)
H₂
Pd(CaCO₃)
Vitamin A ether (XXV)
Toluene (N₂)
p-Toluene-sulfonic acid
(XXIII)
H₂
Pd(CaCO₃)
(XXIV)
Dehydration (p-toluenesulfonic acid) (N₂)
or
(1) PBr₃ or SOCl₂ + pyridine (2) KOH (alcohol)

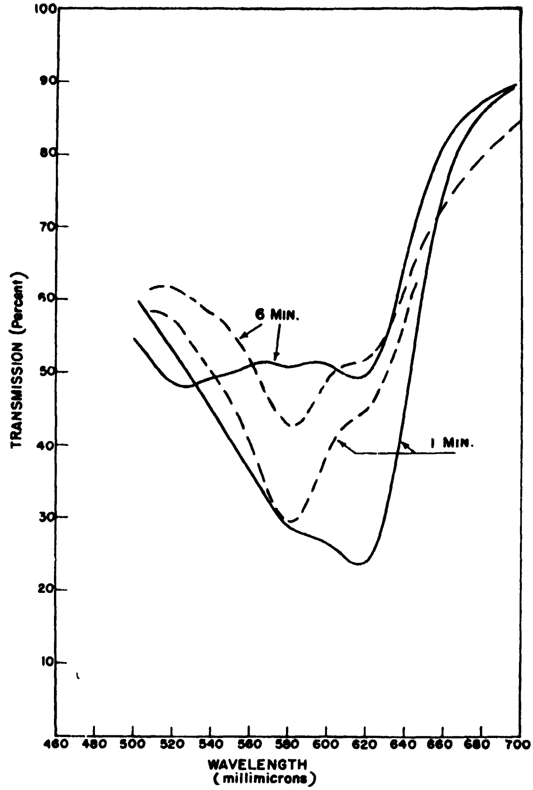


FIG. 2.—Transmission spectra of the antimony trichloride color reaction in chloroform of: (broken line) distilled dehydrobrominated glycol methyl ether (XXI), conc. 0.000394%; (solid line) selectively hydrogenated 5-dehydrovitamin A methyl ether (XXII), conc. 0.000192%, taken by the Hardy color analyzer.

TABLE II

The Effect of High Vacuum Distillation on Methyl Ether of Vitamin A

No. of distillations at 10^{-4}–10^{-5} mm.	λ max. (mμ)	$E_{1\ \text{cm.}}^{1\%}$
No distillation	325	1000
First distillation	325	841
Second distillation	322	570
Third distillation	319	420
Fifth distillation	285–290 (no other band)	
MeOH fractionation of product from third distillation	322	780

During the years 1941 and 1942, the authors made several preparations through the dehydrohalogenation of the glycol ether (XXI, R = methyl, *iso*-propyl, or *tert*-butyl). Many of these were assayed biologically on vitamin A-deficient rats and a summary of the results is presented in Table III. It might be stated here that the glycol (XXI) was tested biologically to find out whether the animal organism would cause dehydration, but the results were negative even when very large doses were fed. Preparation No. 6 was also tested by several other laboratories and all reported appreciable vitamin A activity but not as high as that shown in Table III. Spectroscopically, this sample showed two bands; one at 325 mμ, $E_{1\,\text{cm.}}^{1\%}$ 535, the other at 285 mμ, $E_{1\,\text{cm.}}^{1\%}$ 655 with feeble indications at 345 and 371 mμ, respectively. With antimony trichloride in chloroform, it gave a blue color which exhibited two bands at 580 and at 617 mμ, respectively, the former being more intense. The product is a light yellow oil boiling at 90–95°C. (10^{-5} mm.), having negligible active hydrogen (Zerewitinoff) and an unsaturation equivalent to 5.08 double bonds. The ultimate analysis, however, gave percentages of carbon varying from 1.8 to 2% low. Attempts to purify this product by fractionation at low temperatures were unsuccessful, although the $E_{1\,\text{cm.}}^{1\%}$ (325 mμ) of other samples was raised to 1090 by this method.

The high intensity of the 580 mμ band (Fig. 2, broken line) suggests the possibility that the vitamin A methyl ether as prepared through the above dehydrohalogenation method of (XXI) as well as of (XXIV) is a mixture of the methyl ether and its epoxide. In fact, if one calculates the percentages of carbon and hydrogen in such a mixture from the $E_{1\,\text{cm.}}^{1\%}$ values given above at 285 mμ for the epoxide and at 325 mμ for the methyl ether, one obtains values which agree remarkably well with the actual analyses. This reasoning finds some support in the recent work of Karrer and Jucker (1945d), who found that the chromogen responsible for the 580 mμ band of the Carr-Price reaction is the epoxide of vitamin A (XXVI) and not vitamin A, which is responsible only for the 620 mμ band. In the ultraviolet the vitamin A epoxide, according to Karrer and Jucker, exhibits a maximum at 272 mμ (in chloroform). From their findings, Karrer and Jucker advanced the hypothesis that in fish liver oils as well as in animal liver oils, the vitamin A epoxide coexists with vitamin A, and is formed by the autoöxidation of the latter (Karrer and Jucker, 1945c). The epoxide has also been obtained by treating vitamin A with phthalic acid peracid (Euler, Karrer and Zubris, 1934) in the same manner as epoxides of β- and α-carotenes have been prepared (Karrer and Rutschmann, 1944; Karrer and Jucker, 1945a,b,c). Its structure (XXVI) was predicted by Karrer (1945) who postulated that it exists in equilibrium with its electromer (XXVII). That peracids

TABLE III

Summary of Biological Assays[1]

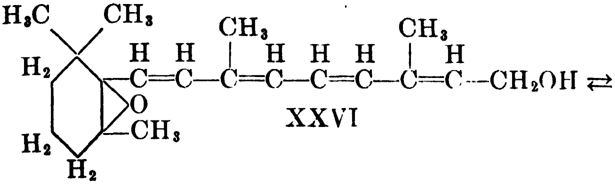
Vitamin preparation	Dose fed per day (γ)	Average gain in wt./rat/28 days (g.)	Remarks[4]
1. MEVA (crude) *via* dehydrochlorination (PCl_3) of (XXI)	331.0[2]	92.0	
2. MEVA (crude) *via* dehydrobromination (PBr_3) of (XXI)	232.0[2]	95.0	
3. Repeat of (2) after standing at 0°C. in olive oil for one month	531.0[2]	40.0	
4. Repeat of (2), new preparation	176.0[2]	25.0	
5. Repeat of (4), simultaneously	190.0[2]	35.0	
6. Same as (2) distilled three times	3.0[2]	14.0	
7. Repeat of (6) after standing at 0°C. in olive oil for one month	6.0[2]	−1.5	All rats survived the test
8. Same as (2) distilled four times	3.0[3]	1.6	One out of eight rats died
9. Repeat of (8)	1.5[3]	7.0	Three out of seven rats died on 6th day of test
10. MEVA (distilled) *via* dehydration of (XXI) with *p*-toluenesulfonic acid as catalyst	189.0[2]	45.0	
11. *iso*-PEVA (distilled) *via* dehydrobromination (PBr_3)	183.0[2]	57.0	
12. Same as (11) distilled twice	3.0[3]	11.0	One out of six rats died on 10th day of test
13. *tert*-BEVA (distilled) *via* dehydrobromination (PBr_3)	7.4[2]	4.0	Six out of ten rats died during test
14. DHMEA (distilled)	111.0[2]	17.0	
15. Same as (14) distilled twice	6.0[2]	19.0	Only two rats were used for this test
16. EEDHHVA (distilled)	96.0[2]	32.0	

MEVA = Methyl ether of vitamin A. *iso*-PEVA = *iso*-Propyl ether of vitamin A. *tert*-BEVA = *tert*-Butyl ether of vitamin A. DHMEA = 5-Dehydromethyl ether of vitamin A. EEDHHVA = 5-Dehydrohomovitamin A ethyl ether.

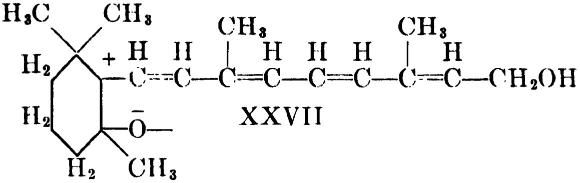
[1] These results were reported to the author during 1941–1942 by Professor Robert S. Harris (M.I.T.).

[2] These samples were prepared in olive oil in which the air was replaced by pure

attack a tertiary-tertiary double bond, like that present in the β-ionone ring, at a greater speed than any other type of double bond, has recently been shown by Swern (1947). This is in agreement with the preferential formation of epoxides at the β-ionone ring when both vitamin A and β-carotene are treated with peracids. In view of these results, the claim (Hunter and Williams, 1945) that vitamin A aldehyde is formed when β-carotene is treated with hydrogen peroxide in glacial acetic acid, should be carefully re-examined.



XXVI



XXVII

The 272 mμ maximum of the vitamin A epoxide, as observed by Karrer and Jucker, seems rather low in view of the presence in the molecule of four conjugated double bonds which should cause an absorption in the region of 285–290 mμ. An autoöxidized sample of pure natural vitamin A which has been kept in methanol under nitrogen for eight years was recently examined spectroscopically and found to have a single band at 285 mμ (Milas, Sakal, Plati *et al.*, 1947). This is in close agreement with the spectrum of the epoxide of the synthetic methyl ether, the structure of which has not yet been definitely established. It also resembles the 580 mμ chromogen of van Eekelen *et al.* (1933) and the subvitamin A of Embree and Shantz (1943) and of Hawkins and Hunter (1944).

---

nitrogen. To each sample was also added 0.1% of hydroquinone based on the vitamin concentration.

[3] These samples were prepared in corn oil in which the air was replaced by pure nitrogen. To each sample was added 0.05% of hydroquinone and 0.05% of lecithin based on the vitamin concentration.

[4] All negative control rats were fed similar doses of olive or corn oil containing only the antioxidants, and died in the first period of the test. All positive control rats were fed 3 U.S.P. units of Reference Cod Liver Oil per day, and showed an average weight increase of 33-44 g./rat/28 days.

Since the original publication of Milas (1945a–o), Isler *et al.* (1946) have also carried out the synthesis of the methyl ether of vitamin A *via* the dehydrohalogenation of the carbinol (XXIV) using phosphorus tribromide to convert the carbinol into the corresponding bromide which was dehydrobrominated with potassium carbonate in acetone. In a more complete publication, Isler *et al.* (1946) used iodine (1.5%) as a catalyst in toluene or ligroin at 95–100°C. to effect the dehydration of the carbinol (XXIV). The crude product, according to the authors, contained three chromogens: one, the vitamin A methyl ether, with a maximum at 325–328 mμ; another with a maximum at 280–290 mμ; and a third, in smaller amounts, with a maximum at 310–315 mμ. The crude product was partitioned between petroleum ether and 95% methanol and the product recovered from the methanol layer retreated with iodine and again partitioned. On the basis of the 325–328 mμ band, the authors claimed a yield of about 25% of vitamin A methyl ether for the two dehydrations and about 20% based on the aldehyde (V). The product recovered from petroleum ether was further purified by chromatography and the methyl ether of vitamin A obtained as a yellow oil boiling at 90–95°C. (10^{-5} mm.). This product had an n_D^{22} of 1.6025 and a single band at 325–328 mμ, $E_{1\,cm.}^{1\%}$ 1415 With antimony trichloride in chloroform it gave a band at 620 mμ, $E_{1\,cm.}^{1\%}$ 1660. When fed to vitamin A-deficient rats by H. Pfaltz and R. Jürgens in doses of 0.8 γ and 1.6 γ, it was found to be equivalent to 1.1 γ and 1.8 γ of β-carotene, respectively. Judging from these results, this preparation was not 100% pure vitamin A methyl ether, since the latter has been prepared recently from natural vitamin A by Hanze *et al.* (1946) who reported the following properties: a light yellow solid, m.p. 33–34°C. having an $E_{1\,cm.}^{1\%}$ (326 mμ) of 1660 and a biological potency of about 3,000,000 U.S.P. XXII units/g.

More recently, Milas, Sakal *et al.* (1947) developed another method of dehydrobromination in which the glycol (XXI) or the carbinol (XXIV) (R = ethyl) were treated at 100°C. with pyridine hydrobromide in the presence of excess pyridine, and the resulting product treated further with hot alcoholic potash. The crude product (80–90% yield) showed a broad band in the ultraviolet of very high intensity between 300 and 370 mμ and one of low intensity at 285–290 mμ and gave a deep blue color with antimony trichloride in chloroform. When partitioned between equal volumes of 83% ethanol and petroleum ether, most of it went into the petroleum ether layer which was chromatographed on activated alumina. The greater part of the product passed through the alumina unadsorbed. This portion was fractionated through a molecular still of the falling film type at 10^{-5} mm. and the largest fraction (80%), a light yellow oil, was analyzed spectroscopically. It was found to have a fine structure of three bands in the ultraviolet (Fig. 3); one at 330 mμ, $E^{1\%}_{1\,\text{cm.}}$ 1690, a second at 348 mμ, $E^{1\%}_{1\,\text{cm.}}$ 1830 and a third at 367 mμ, $E^{1\%}_{1\,\text{cm.}}$ 1520. The shape and position of these bands are identical with those obtained recently by Shantz (1946) for the hydrocarbon

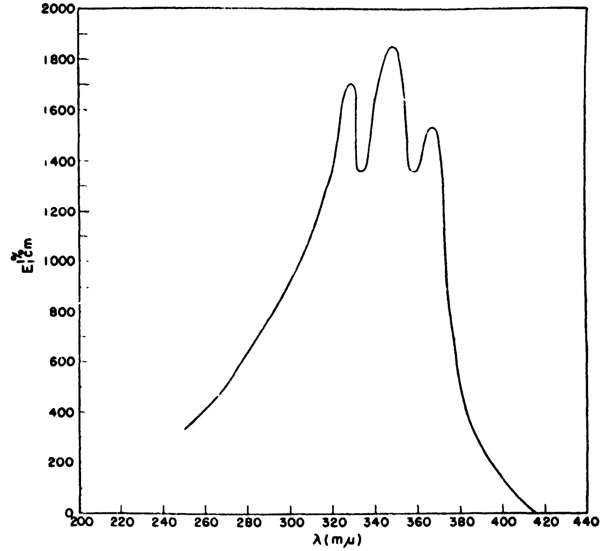
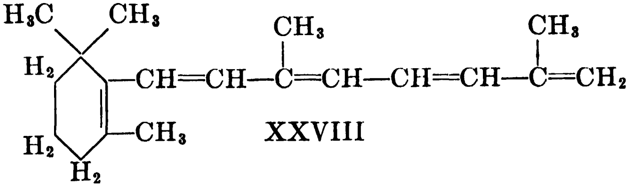


FIG. 3.—Absorption spectrum in ethanol of "*allo*-vitamin A ethyl ether."

(XXVIII). That this substance was not a hydrocarbon was shown by the fact that it still possessed the ethoxyl group, and molecular weight



XXVIII

determinations and hydrogenation gave values in remarkable agreement with those expected for the vitamin A ethyl ether, although carbon and hydrogen analyses were slightly lower than the theoretical. On standing under nitrogen at 0°C. for over six months, it partially crystallized into light yellow crystals which melted at about 28–30°C. Upon ozonization it yielded geronic acid (as 2,4-dinitrophenylhydrazone) indicating the presence in the molecule of the β-ionone ring. No biological results are as yet available for this substance. In view of the peculiar nature of the ultraviolet spectrum, one cannot state unequivocally at present that this substance is the normal ethyl ether of vitamin A, in spite of the fact that other evidence seems to point strongly to this conclusion. For this reason, the term "*allo*vitamin A ethyl ether" is suggested.

Sample No. 10 shown in Table III was prepared by the dehydration in toluene of the glycol (XXI) using catalytic amounts of *p*-toluenesulfonic acid. When distilled from a shallow vessel at 10^{-5} mm., the distillate exhibited both the 325 and the 285–290 mμ bands and gave a blue color with antimony trichloride in chloroform. Hydrogenation showed the presence of 4.98 double bonds and a Zerewitinoff determination showed negligible active hydrogen.

Vitamin A methyl ether was also synthesized [Milas (1946); Milas, Sakal *et al.* (1947)] by the selective hydrogenation of 5-dehydrovitamin A methyl ether (XXII) which was prepared by the dehydration of the glycol (XX, R = methyl) using catalytic amounts of *p*-toluenesulfonic acid. The crude 5-dehydrovitamin A methyl ether was purified by partitioning between petroleum ether and 95% methanol followed by low temperature fractionation and molecular distillation. The ultraviolet spectrum of the purest specimen obtained is shown in Fig. 4, curve A. Although the crude product showed two broad bands, one at 310–330 mμ and the other at 280–290 mμ, the purified product showed a single maximum at 322 mμ, $E^{1\%}_{1\,\text{cm.}}$ 1600, and gave the expected unsaturation. Upon ozonization it yielded geronic acid, indicating the presence in the molecule of the β-ionone ring. When one mole of hydrogen was added to it in the presence of 1% palladium deposited on calcium carbonate, and the product purified by low temperature fractionation in methanol followed by molecular distillation, a specimen [yellow oil, b.p. 90–95°C. (10^{-5} mm.)] was obtained which had an ultraviolet spectrum [$E^{1\%}_{1\,\text{cm.}}$ (323 mμ) 1560, Fig. 4, curve B] which was very similar to that of the 5-dehydrovitamin A methyl ether. The spectrum of the antimony trichloride color reaction in chloroform is shown in Fig. 2 (solid line curves) taken one minute and six minutes after mixing, respectively. The one minute curve shows a principal maximum at 618 mμ, $E^{1\%}_{1\,\text{cm.}}$ 3284. Ozonization of this vitamin A ether gave geronic acid, again indicating the presence of the β-ionone nucleus.

5-Dehydrovitamin A ethyl ether (XXII, R = ethyl) was also synthesized by the dehydration of either the glycol (XX) or the carbinol (XXIII) in the presence of *p*-toluenesulfonic acid. The crude product had similar properties to the corresponding 5-dehydrovitamin A methyl ether and was purified by the same procedure. The purest specimen obtained had the ultraviolet absorption spectrum shown in Fig. 4, curve C. Selective catalytic hydrogenation did not appreciably change the shape of the band or the position of the maximum.

Since it is well known (Zechmeister, 1944; Campbell and Campbell,

1942) that selective catalytic hydrogenation of an acetylene leads predominantly to a *cis* olefin (Campbell and Edy, 1941), and chemical reduction usually leads to a *trans* olefin, Milas, Sakal *et al.* (1947) studied several chemical methods for the conversion of 5-dehydrovitamin A ethyl ether into vitamin A ethyl ether. The ultraviolet absorption spectrum and unsaturation were taken before and after reduction. Of all the methods studied, zinc dust in 90% alcoholic potassium hydroxide was found the most effective. A product was obtained which, after purification by low temperature fractionation from methanol, had the ultraviolet spectrum shown in Fig. 4, curve D. The product also showed an unsaturation of 4.85–5.2 double bonds as compared with the original of 6.0–6.1 double bonds. Although the intensity of the ultraviolet maximum was increased, an increase which might be due to further purification, the position of the maximum was essentially the same.

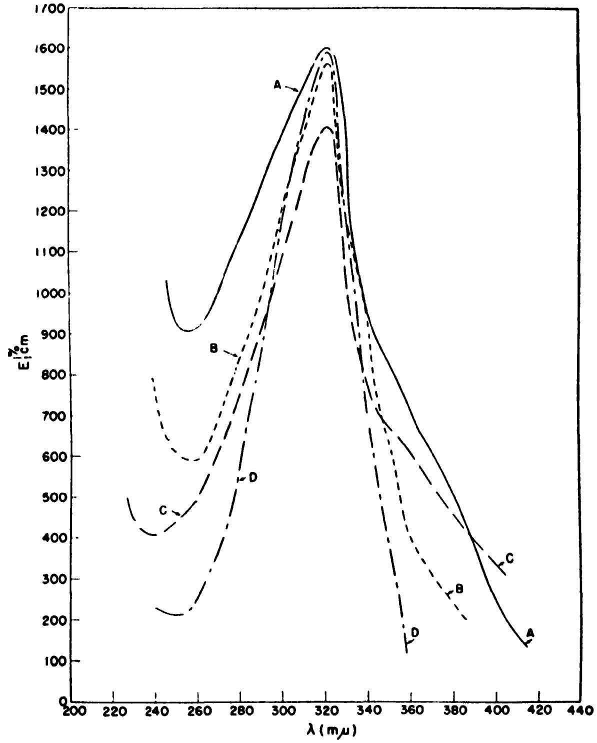


FIG. 4.—Absorption spectra in ethanol of: (A) 5-dehydromethyl ether of vitamin A from XX; (B) methyl ether of vitamin A *via* partial hydrogenation of XXII; (C) 5-dehydrovitamin A ethyl ether *via* dehydration of either XX or XXIII; (D) ethyl ether of vitamin A by reduction of XXII using zinc dust and alkali.

Moreover, the shape of the absorption curve is somewhat the same as that obtained from the catalytically hydrogenated 5-dehydrovitamin A methyl ether (curve B).

A summary of the spectroscopic data of the important compounds discussed in this Section is given in Table IV.

Impure samples of both the methyl and ethyl ethers made by the partial catalytic hydrogenation of the 5-dehydroethers of vitamin A (XXII) were found biologically active when tested orally in vitamin A-deficient rats. For example, samples with an $E^{1\%}_{1\,\mathrm{cm.}}$ (320–323 mμ) of about 400–500 gave potencies of the order of 100,000 U.S.P. units/g. The comparison was made against Reference Cod Liver Oil. The final purified products have not yet been tested biologically.

TABLE IV

Summary of Ultraviolet Absorption Spectra of Ethers and 5-Dehydroethers of Vitamin A (in alcohol)

Vitamin A ether	λ max. (mμ)	$E^{1\%}_{1\,\mathrm{cm.}}$	$\epsilon_{\mathrm{mol.}}$	log $\epsilon_{\mathrm{mol.}}$
DHMEA (Fig. 4, curve A)[1]	322	1600	47,680	4.68
MEVA (Fig. 4, curve B)[2]	323	1560	46,800	4.67
MEVA[3]	325	1090		
MEVA[4]	325–328	1415	42,450	4.63
MEVA (natural)[5]	326	1660	49,800	4.70
DHEEA (Fig. 4, curve C)[6]	322	1410	43,992	4.64
EEVA (Fig. 4, curve D)[7]	323	1590	49,926	4.70
EEVA[8]	325–327	1500	47,100	4.67
EEVA (*allo*)[9]	330	1690	53,066	4.70
	348	1830	57,462	4.76
	367	1520	47,728	4.68

[1] 5-Dehydromethyl ether of vitamin A from XX.

[2] Methyl ether of vitamin A from XXII.

[3] Methyl ether of vitamin A *via* dehydrobromination of either XXI or XXIV purified by fractionation at temps. between 0° and −78°C. This sample exhibits also the 285–290 mμ band.

[4] The Swiss vitamin A methyl ether made *via* dehydrohalogenation of XXIV and purified by chromatography. (Isler, Kofler *et al.* 1946; Isler, Huber *et al.* 1946.)

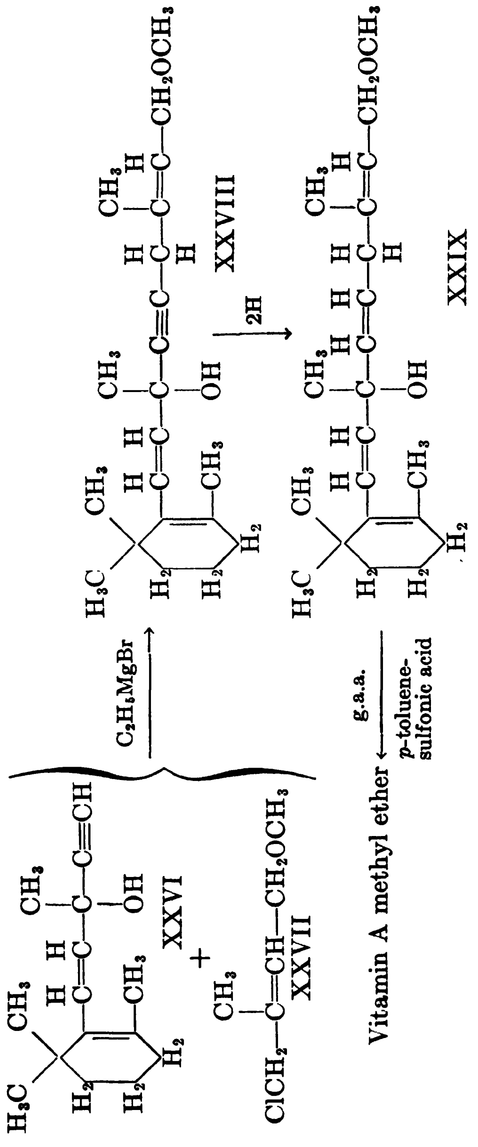
[5] Methyl ether prepared from natural vitamin A. (Hanze *et al.* 1946.)

[6] 5-Dehydroethyl ether of vitamin A prepared by dehydration of either XX or XXIII purified *via* low temperature fractionation and chromatography + molecular distillation.

[7] Ethyl ether of vitamin A from XXII *via* reduction with Zn dust + alkali.

[8] Ethyl ether of vitamin A *via* dehydration of the carbinol (XXIV) with *p*-toluene sulfonic acid, purified by low temperature fractionation.

[9] Ethyl ether of vitamin A (*allo*) *via* the dehydrobromination (pyridine hydrobromide) of either XXI or XXIV purified by low temperature fractionation + chromatography + molecular distillation.


XXVI
+
XXVII
C_2H_5MgBr
XXVIII
2H
XXIX
g.a.a.
p-toluene-sulfonic acid
Vitamin A methyl ether

In a preliminary report Oroshnik (1945) has also claimed the synthesis of vitamin A methyl ether by a slightly different scheme. The acetylene carbinol (XXVI) (Gould and Thompson, 1935; Davies *et al.* 1935; Rivers, 1941) was treated with ethyl magnesium bromide and the acetylene Grignard formed reacted with 2-methyl-4-methoxy-1-chlorobutene-2 (XXVII) to form the methoxyacetylenecarbinol (XXVIII). This was catalytically hydrogenated to the methoxycarbinol (XXIX) which was dehydrated in glacial acetic acid with *p*-toluenesulfonic acid to give vitamin A methyl ether. Upon distillation of the crude product, the author obtained two fractions: one boiling at 100–103°C. (10^{-3} mm.) and having an $\epsilon_{mol.}$ (312 mμ) value of 27,470; the other boiling at 110–115°C. (10^{-3} mm.) and having an $\epsilon_{mol.}$ (315 mμ) value of 33,750. Obviously from these results neither product appears to be pure vitamin A methyl ether.

Furthermore, it has not been definitely established whether this vitamin A methyl ether is identical with that prepared by Milas, Sakal *et al.* (1947) or by Isler, Huber *et al.* (1946) in view of the fact that both ozonization experiments to prove the presence of the β-ionone ring and biological experiments are lacking. Isler, Huber *et al.* (1946) pointed out, however, that it is quite possible that the methyl ether of Oroshnik is a stereoisomer of vitamin A methyl ether. It is the opinion of the present author that if Oroshnik's sample contained vitamin A methyl ether, further purification would produce a pure specimen which would have the same absorption spectrum as that of the synthetic methyl ether of Milas, Sakal *et al.* (1947) and Isler, Huber *et al.* (1946).

IV. SYNTHESIS OF VITAMIN A ESTERS

The synthesis of vitamin A esters and their subsequent hydrolysis to vitamin A were also studied by Milas (1945m) and Milas, Sakal, Grossi and Pitt (1947). Two of the esters studied were the benzoate and the acetate. When the Grignard of the acetylene carbinol (XIX) was added to the 4-benzoxy- or acetoxybutanone-2 (XXX), the glycols (XXXI) were produced in yields of 80–90%. Purification of these glycols was accomplished by first removing the unconverted acetylene carbinol as its silver salt followed by partitioning the remaining product between petroleum ether and 90% methanol and recovering the glycol esters from the methanol layer. Further purification was affected by low temperature fractionation from petroleum ether. Their selective hydrogenation proceeded smoothly with high yields. When the glycol acetate (XXXII) was treated at 0°C. with thionyl chloride in a pyridine-ether mixture, the corresponding dichloride (XXXIII) was formed, together with a small portion of the completely dehydrochlorinated acetate in a yield of 75–80%. This dichloride gave a deep blue color with antimony trichloride in chloroform and had an $E^{1\%}_{1\,\text{cm.}}$ (325–328 mμ) value of 220. The dichloride without further purification was dehydrochlorinated with alcoholic potash at 60–80°C. The crude product thus obtained had an $E^{1\%}_{1\,\text{cm.}}$ (325–327 mμ) value of 673 and an additional maximum at 280–290 mμ with feeble inflections at 345 and 366 mμ, respectively. Although this product was completely free from chlorine and acetate groups, the dehydrochlorination must have produced two products as in similar experiments in the synthesis of vitamin A ethers. The crude product was biologically active in doses of about 20–30γ. Attempts to separate the vitamin A from the 280–290 mμ chromogen at low temperatures from methanol, ethyl formate or petroleum ether were not successful, although a product was obtained which had an $E^{1\%}_{1\,\text{cm.}}$ (325 mμ) value of 1174 and a similar value at 287 mμ (see Fig. 5). In view of the premature liquidation of our project after the war, the chromatographic method which was so successful in the purification of the synthetic vitamin A ethers was not tried. The glycol benzoate (XXXII, R = COC_6H_5) was also dehydrochlorinated with no better results.

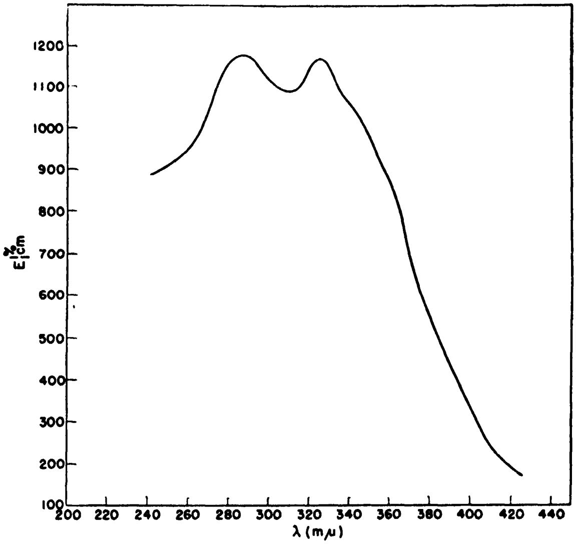
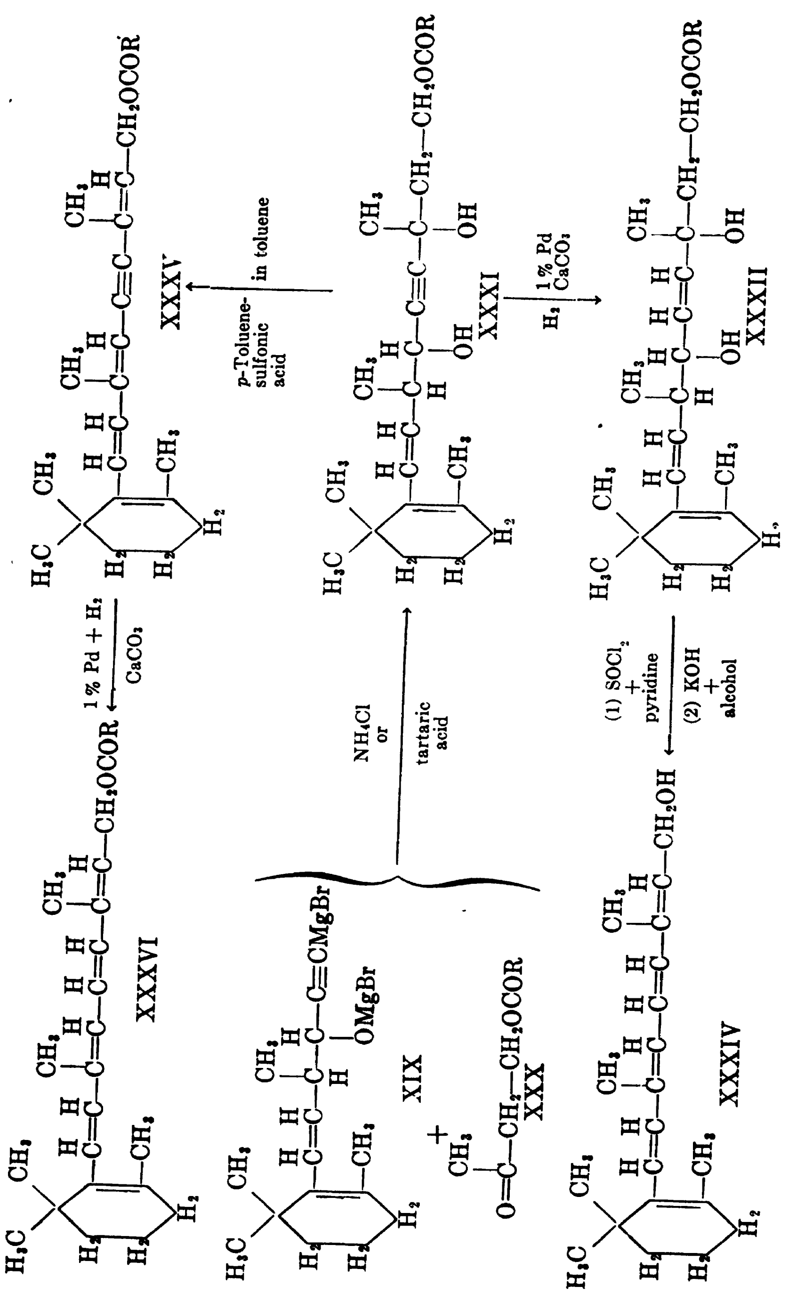


FIG. 5.—The ultraviolet absorption spectrum in ethanol of dehydrochlorinated glycol acetate (XXXII).

The direct dehydration of the 5-dehydroglycolesters (XXXI) with catalytic amounts of *p*-toluenesulfonic acid was also studied, and the corresponding 5-dehydroesters (XXXV) were obtained in 75–85% yields. The purification of these esters was accomplished by low tem-


XXXVI
1 % Pd + H₂
CaCO₃
XXXV
p-Toluene-sulfonic acid
in toluene
XIX
+
XXX
NH₄Cl or tartaric acid
XXXI
H₂ 1 % Pd CaCO₃
XXXII
(1) SOCl₂ + pyridine
(2) KOH + alcohol
XXXIV

perature fractionation in methanol. The purest 5-dehydrovitamin A acetate obtained by this method of purification had an $E^{1\%}_{1\,cm.}$ (322 mμ) value of 1250, while the corresponding 5-dehydrovitamin A benzoate had an $E^{1\%}_{1\,cm.}$ (322 mμ) value of 1042. In addition to the 322 mμ band, the 280–290 mμ band was also present in the ultraviolet spectra of these esters. In view of this fact, their absolute purity is questioned.

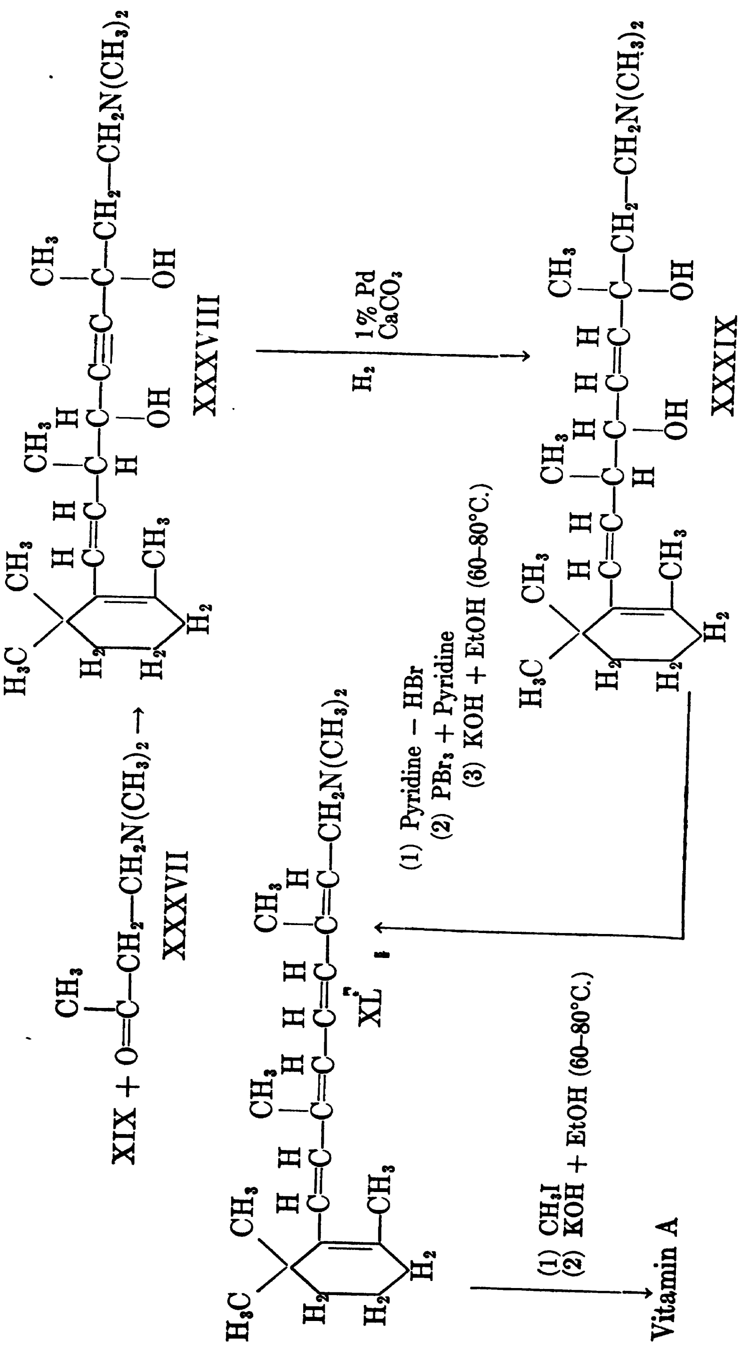
The selective hydrogenation of the 5-dehydrovitamin A benzoate was effected in the presence of 1% palladium deposited on calcium carbonate. As in the case of the partial hydrogenation of the 5-dehydroethers, there was a slight lowering in the intensity of the maximum but there was little or no change in its position.

V. DIMETHYLAMINOVITAMIN A AND ITS CONVERSION TO VITAMIN A

The synthesis of vitamin A by this method is based on the fact that upon heating trimethyl alkyl quaternary salts or bases in which the alkyl group contains no loosely bound hydrogen atoms on the carbon β to the quaternary nitrogen, decomposition occurs in such a manner as to yield chiefly trimethylamine and an alcohol. For example, trimethylallylammonium hydroxide gives, on heating, chiefly trimethylamine and allyl alcohol. On the basis of this reasoning, Milas (1947) and Milas, Sakal, Plati and Yamins (1947) have obtained vitamin A by the exhaustive methylation of dimethylaminovitamin A (XL).

In this synthesis the Grignard of the acetylenic carbinol (XIX) was added to 4-dimethylaminobutanone-2 (XXXVII) and the dimethylamino acetylenic glycol (XXXVIII) was obtained in about 30% yield. This was selectively hydrogenated to the glycol (XXXIX), which was converted at room temperature into the hydrobromide by means of pyridine hydrobromide. When the hydrobromide was treated in a pyridine-benzene mixture at 0°C. with phosphorus tribromide and the dibromide formed dehydrobrominated with alcoholic potash at 60–80°C., the dimethylaminovitamin A (XL) was formed in about 80% yield. This gives a broad band in the ultraviolet between 300 and 330 mμ and a purplish-blue precipitate with antimony trichloride in chloroform. Further purification was effected by fractionation at low temperatures from petroleum ether. The purest specimen obtained had an $E^{1\%}_{1\,cm.}$ (323–324 mμ) value of about 1200. The present author has been informed recently that Merck and Co. have repeated the synthesis of this compound and found that it exhibited an appreciable biological activity when fed to vitamin A-deficient rats (private communication).

The conversion of the dimethylaminovitamin A into vitamin A was accomplished by first preparing the methiodide, then converting the latter into the hydroxide with alcoholic potash, and heating the hydroxide


XIX + O=C(CH3)—CH2—CH2N(CH3)2 →
XXXVII
XXXVIII
H2 1% Pd CaCO3
XXXIX
(1) Pyridine – HBr
(2) PBr3 + Pyridine
(3) KOH + EtOH (60–80°C.)
XL
(1) CH3I
(2) KOH + EtOH (60–80°C.)
Vitamin A

at 60–70°C. A product was obtained by this procedure which was free from nitrogen. This was found to have an ultraviolet absorption spectrum with a broad band between 300 and 330 mμ, having an $E^{1\%}_{1\,\mathrm{cm.}}$ (322–323 mμ) value of 604. With antimony trichloride it gave a deep blue color which exhibited a strong maximum at 620 mμ and a weaker one at 580 mμ. In an exploratory experiment, this crude product was fed to vitamin A-deficient rats in doses of 52 γ/day. An average weight increase of 29 g./rat/28 days was obtained (R. S. Harris). All negative control rats died in the early part of the testing period. On further purification of the crude sample by low temperature fractionation in methanol, a specimen was obtained which had an $E^{1\%}_{1\,\mathrm{cm.}}$ (324 mμ) value of 1412.

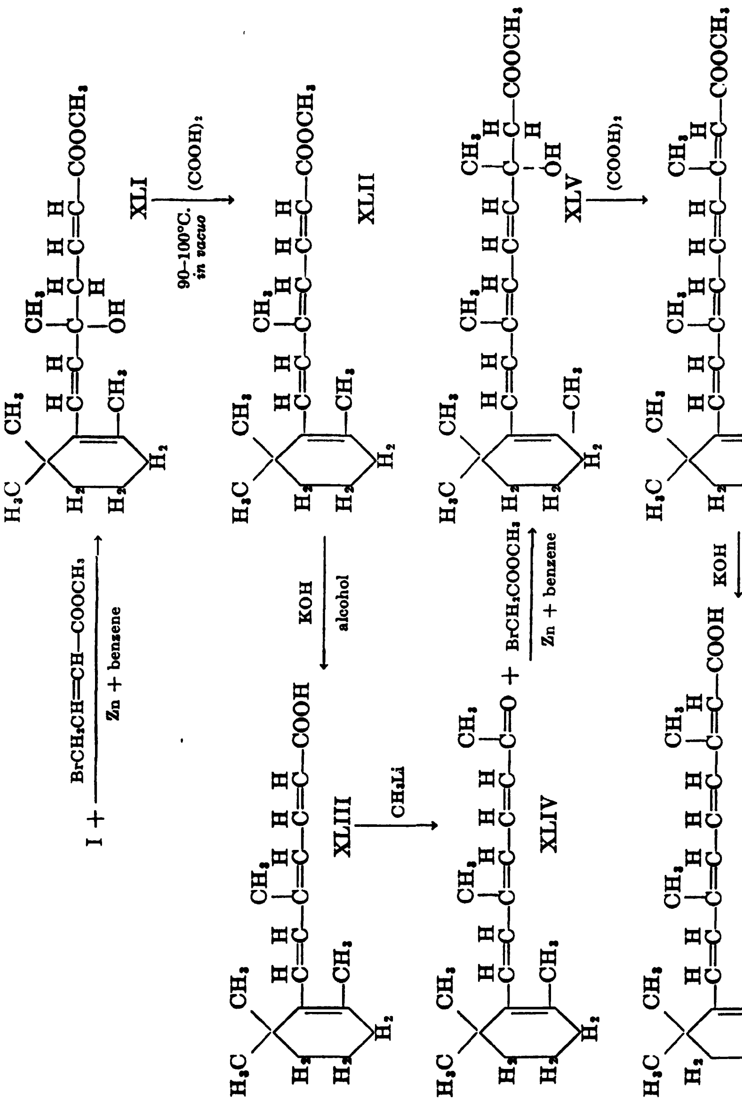
The preparation of vitamin A without isolation of the dimethylaminovitamin A was also tried with less satisfactory results. The glycol (XXXIX) was converted to the methiodide and the latter dehydrobrominated using first phosphorus tribromide in pyridine, followed by treatment with alcoholic potash at 60–70°C. This process produced a mixture free from nitrogen, but its spectrum contained two bands: one of high intensity with a maximum at 305 mμ and a secondary band with a maximum at 321 mμ. With antimony trichloride in chloroform, this product gave a deep blue color which exhibited a maximum of higher intensity at 620 mμ than at 580 mμ. In this process it is quite possible that loss of trimethylamine occurred before complete dehydrobromination, thereby producing a substance with only four double bonds in conjugation. This may explain the shift of the maximum from 324 mμ to 305 mμ. The processes discussed under this section were also developed during the war and further investigation was made impossible by the liquidation of the project.

VI. SYNTHESIS OF "VITAMIN A ACID"

In addition to the biologically active vitamin A products described in the foregoing sections, "vitamin A acid" (XLVII) has been synthesized and found to be biologically active. The synthesis of this acid was originally claimed by Heilbron *et al.* (1936) from β-ionylidene acetaldehyde. Subsequently (Sobotka *et al.* 1943; Karrer and Rüegger, 1944; Milas and Sakal, 1947) however, it was not found possible to obtain β-ionylidene acetaldehyde by the method used by these authors, and this synthesis of the vitamin A acid is therefore highly doubtful. More recently, Arens and van Dorp (1946) have synthesized the vitamin A acid by the following series of reactions: β-ionylidene crotonic acid (XLIII) was synthesized by a method originally attributed to Ziegler (1947) who used the Reformatsky reaction to condense β-ionone (I) with γ-bromocrotonic methyl ester (Ziegler, Spath *et al.* 1942). Although Ziegler assumed that he obtained the dehydrated ester (XLII), van Dorp and Arens obtained a mixture of esters which contained predominantly the hydroxy ester (XLI), the dehydration of which was best effected by anhydrous oxalic acid under reduced pressure at 90–100°C. Karrer, Jucker and Schick (1946) effected the same dehydration by means of phenyl isocyanate. The yields of the ester were low, and a considerable amount of "β-ionone" was recovered unchanged. The low yields obtained in this step were not surprising in view of the results of Fuson *et al.* (1938), who reported that Reformatsky reactions with γ-bromocrotonic esters and ketones or aldehydes give low yields of the expected products. The ester (XLII) was then saponified with alcoholic potash and the acid (XLIII) recovered and crystallized. A yield of slightly over 8% was obtained. In an independent synthesis of this acid, Heilbron *et al.* (1946a,b) reported a yield of 15%. This acid has a m.p. of 155.5–157.5°C. (160°C., Heilbron *et al.*, 1946b) and an ultraviolet absorption maximum at 323 mμ, log ε 4.56. The C_{18} ketone (XLIV) was obtained in a yield of 90% by treating the free acid with two moles of lithium methyl following the method of Gilman and van Ess (1933). Heilbron *et al.* (1946a,b) obtained the same ketone by treating β-ionylidene crotonyl chloride with cadmium dimethyl, while Karrer, Jucker and Schick (1946) treated the same chloride with zinc methyl iodide. The ketone was a yellow oil which formed a semicarbazone, m.p. 188.6–189.6° which had an absorption maximum at 349 mμ, log ε 4.69. It also gave a wine-red color with antimony trichloride in chloroform.

By means of a second Reformatsky, this time with methyl bromoacetate, the C_{18} ketone was converted into a mixture of esters (XLV) and (XLVI) which was dehydrated with oxalic acid (Karrer, Jucker and Schick, 1946, used phenylisocyanate) and the resulting product saponified with alcoholic potash. An orange oil was obtained from which the vitamin A acid was crystallized as yellow crystals (m.p. 181.5°C.) in a yield of about 9% based on the C_{18} ketone used. The vitamin A acid has an ultraviolet absorption maximum at 347 mμ, log ϵ_{max} 4.64 and, with antimony trichloride in chloroform, it gives a red color with a purplish lustre, having an absorption maximum according to Karrer *et al.* at 572 mμ. When fed to vitamin A-deficient rats in peanut oil, 4 γ was found to be equivalent to one international unit. When an aqueous solution of its sodium salt was injected subcutaneously, the potency was greatly increased, 1 international unit being equivalent to 0.6 γ.

Using somewhat similar procedure, Karrer, Jucker and Schick (1946) have synthesized the "vitamin A acid" as well as the corresponding acid from α-ionone, but gave no biological results.

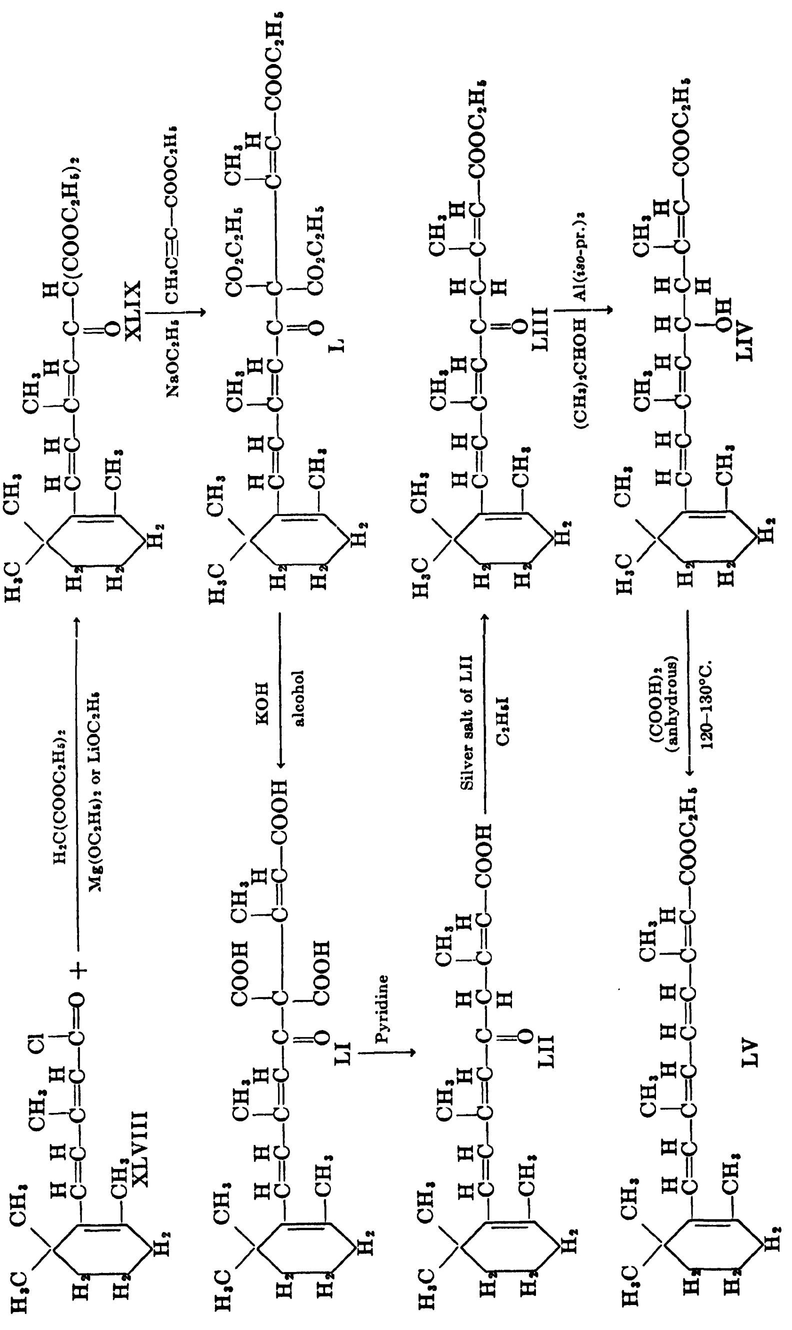


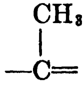
I +
BrCH₂CH=CH—COOCH₃
Zn + benzene
XLI
90–100°C. in vacuo
(COOH)₂
XLII
KOH
alcohol
XLIII
CH₃Li
XLIV
BrCH₂COOCH₃
Zn + benzene
XLV
(COOH)₂
XLVI
KOH
alcohol
XLVII

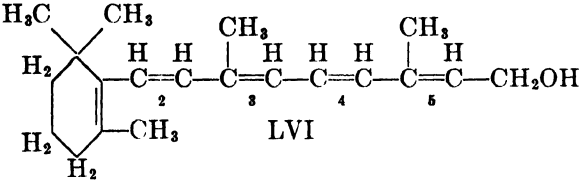
An alternative procedure for the synthesis of the "vitamin A acid" has been developed by Milas (1945c) and Milas, Edgerton *et al.* (1947). In this procedure, β-ionylidene acid chloride (XLVIII) (Karrer *et al.* 1932) was condensed smoothly with diethyl malonate in the presence of either magnesium or lithium alcoholate to give the keto diester (XLIX) in a yield of 85%. This ester exists entirely in the enolic form, since it gives a green color with ferric chloride and a prominent band in the ultraviolet with a maximum at 329 mμ. The condensation of this ester with ethyl tetrolate was also a smooth reaction giving the ketotriester (L) in a yield of 78%. This keto triester was also prepared in good yields by condensing β-methyl-γ-carboethoxyglutaconic ester with β-ionylidene acid chloride in the presence of sodium ethoxide as condensing agent. The keto triester gave a negative ferric chloride reaction and an ultraviolet absorption maximum at 280 mμ. The keto triester was then saponified with alcoholic potash and the keto tricarboxylic acid (LI) recovered in 80% yield. Owing to the instability of this acid, it was never obtained entirely free from the keto dicarboxylic acid. Several methods were studied for the partial decarboxylation of the keto tricarboxylic acid into the keto acid (LII). Of these, the use of pyridine at room temperature gave the best results (90% yield). This keto acid analyzed well and gave a prominent band in the ultraviolet with a peak at 286 mμ. No attempt was made to crystallize it, although it should exist in a crystalline form. This keto acid has the skeleton structure of vitamin A as well as that of "vitamin A acid." To obtain the "vitamin A ethyl ester" (LV), the keto acid was first esterified by treating its silver salt with ethyl iodide, or in absolute ethanol in the presence of anhydrous copper sulfate and small amounts of concentrated sulfuric acid. A yield of 76% of the keto ester (LIII) was obtained. The keto ester was then reduced with aluminum isopropoxide in isopropyl alcohol (Lund, 1937), and the hydroxy ester was isolated in yields of 70–75%. The dehydration of the hydroxy ester was not a smooth reaction, and on several occasions the product was recovered unchanged. For example, anhydrous oxalic acid in toluene at 110°C. gave very little or no dehydration. However, when the hydroxy ester was treated with either anhydrous oxalic or *p*-toluenesulfonic acid at 120–130°C. under a highly reduced pressure, the ester (LV) was obtained in about 50% yield. This ester had a broad band in the ultraviolet between 300 and 360 mμ with a peak at about 345–346 mμ. No attempt was made to obtain the "vitamin A acid" from this ester, nor was the latter tested biologically.

VII. STEREOCHEMISTRY OF VITAMIN A

No discussion of the synthesis of vitamin A and related products would be complete without including the stereochemistry of these prod-


XLVIII
H₂C(COOC₂H₅)₂
Mg(OC₂H₅)₂ or LiOC₂H₅
XLIX
NaOC₂H₅
CH₃C≡C—COOC₂H₅
L
KOH
alcohol
LI
Pyridine
LII
Silver salt of LII
C₂H₅I
LIII
(CH₃)₂CHOH
Al(iso-pr.)₃
LIV
(COOH)₂
(anhydrous)
120–130°C.
LV

ucts. For a long time the organic chemists assumed that in polyenes which contain more than three double bonds in conjugation, the stereochemical problem is minimized or vanishes altogether, due to the mutual interaction of the double bonds or the increased mobility of the valence electrons (Wittig and Wiemer, 1930; Coulson, 1939; Wittig and Fartmann, 1939). The evidence which has accumulated during the last seven years tends to discredit this view. Pauling (1939), and later Zechmeister *et al.* (1941) and Zechmeister (1944), have concluded that in carotenoids, which are made up of dehydrogenated isoprene units, the number of stereoisomers of a given carotenoid depends upon the number of "stereochemically effective" double bonds it contains. The stereochemically ineffective conjugated double bonds are those located in a ring and those which are sterically hindered and are usually found adjacent to  $\overset{CH_3}{\overset{|}{—C=}}$ groups. On the basis of this view, vitamin A (LVI) has two "stereochemically effective" double bonds, 3 and 5, and should exist in four stereoisomeric forms; *trans-trans*, *trans-cis*, *cis-trans*, and *cis-cis*.



LVI

Since the *trans* configuration is the most stable configuration, all natural carotenoids, according to Zechmeister (1944), have, as a rule, an all-*trans* configuration. Spectroscopically the *cis* isomers absorb at shorter wave lengths (Zechmeister, 1944) and have lower extinction coefficients (Koch, 1942) than the *trans* isomers. The melting points of the *cis* isomers are usually lower than those of the *trans* isomers. Addition of maleic anhydride to *cis* isomers is slower than to *trans* isomers (Morrell *et al.*, 1942). Finally, Deuel *et al.* (1946) reported recently that the provitamin activity of neocryptoxanthin A toward the rat was 71% of that of all-*trans* cryptoxanthin and 42% of that of all-*trans*-β-carotene.

Of the four theoretically possible stereoisomers of vitamin A only two have so far been identified; vitamin A, which is assumed to have an all-*trans* configuration, and the recently discovered neovitamin A, which has been assigned a 3-*trans*-5-*cis* configuration by Robeson and Baxter (1945; 1947). These investigators have found that neovitamin A constitutes about 35% of the total vitamin A content of the common fish liver oils. A comparison of the properties of the two vitamins is given in Table V.

TABLE V

Comparison of Vitamin A with Neovitamin A
(Robeson and Baxter (1947))

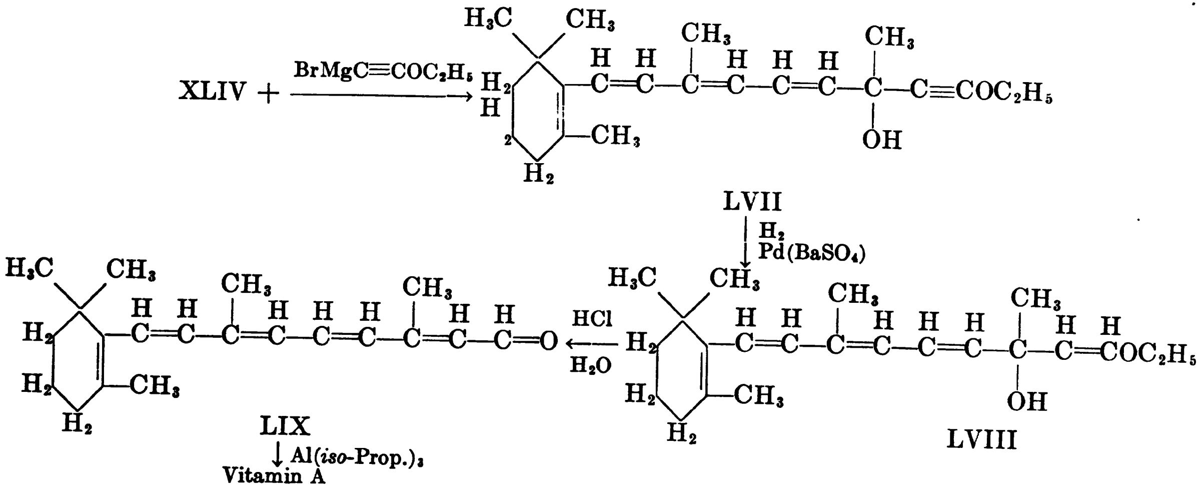
	Vitamin A	Neovitamin A
Crystal form	Yellow prisms	Yellow needles
M.p.	62–64°C.	58–60°C.
M.p. of phenylazobenzoate	79–80°C.	94–96°C.
M.p. of anthraquinone-β-carboxylate	121–122°C.	134–136°C.
λ_{max}	324–325 mμ	328 mμ
$E_{1\,cm}^{1\%}$	1740 (325 mμ)	1645 (328 mμ)
Resistance to atmospheric oxidation	Less resistant	More resistant
Rate of maleic anhydride addition	Rapid	Slow
Rate of dehydration with alcoholic hydrogen chloride	Rapid	Slow

The biological potency of neovitamin A is apparently the same as that of vitamin A. This has been ascribed to the interconversion *in vivo* of neovitamin A to vitamin A. This interconversion has also been accomplished *in vitro* by the catalytic action of iodine.

In the synthesis of vitamin A or its derivatives described in the foregoing sections, the double bonds 3 and 5 were formed either by the elimination of water or halogen acids. Although there is ample evidence in the literature (Winstein *et al.*, 1939; Huckel *et al.*, 1940; Young *et al.*, 1943; Cristol, 1947) to show that the elimination of water or halogen acids is a *trans* elimination, it does not always lead exclusively to the formation of *trans* double bonds. It may or may not be significant in this connection that the dehydration of 5-dehydroacetylene glycols or 5-dehydrocarbinols described under Sections III and IV and subsequent partial hydrogenation of the resulting polyvinyl acetylenes led always to final products which absorbed at shorter wave lengths than those produced by the dehydrohalogenation of either the partially hydrogenated 5-dehydroglycols or 5-dehydrocarbinols. Is it possible, therefore, that both *cis* and *trans* synthetic vitamin A derivatives or mixtures of these were actually obtained? This problem has not been cleared in any of the cases studied. Although biologically-active vitamin A and its derivatives have been synthesized, in no case has a synthetic product been shown to be identical in *every respect* with the natural product. Furthermore, it may be possible that future work will show that even the natural vitamin A is not a homogeneous substance. The recent work of Robeson and Baxter (1947) has already made a beginning in this direction.

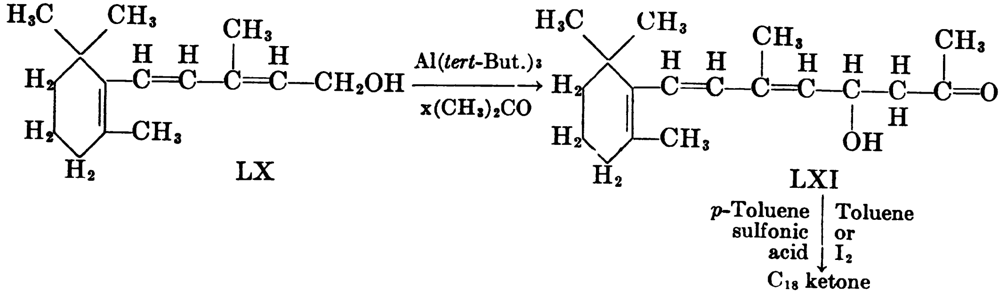
ADDENDUM

Since the original writing of this review, van Dorp and Arens (1947) published a new synthesis of vitamin A which is illustrated by the following reactions:



The aldehyde LIX was found to be identical with that prepared by Hawkins and Hunter (1944). In the last step the authors claim a yield of 35% of vitamin A as determined spectroscopically and by its biological activity.

A new and simplified synthesis of the C_{18} ketone (XLIV) was published recently by Milas and Harrington (1947). This ketone was synthesized by the application of the Oppenauer oxidation [Batty, Buraway *et al.* (1938)] on β-ionylidene ethyl alcohol (LX) which was prepared in good yields by the reduction of ethyl β-ionylidene acetate with lithium aluminum hydride [Finholt, Bond, and Schlessinger (1947)]. The yields in both steps were good and the whole method promises an important simplification of the synthesis of vitamin A itself. The authors proposed a direct application of the method with lithium aluminum hydride to vitamin A ester (LV) to produce vitamin A. This method would simplify the entire synthesis of vitamin A and may make possible its production more economically.



A crystalline synthetic vitamin A has also been announced recently by Cawley, Robeson *et al.* (1947). Two crystalline esters, the acetate and the anthraquinone carboxylate, have been prepared from it and found to be identical with those prepared from natural vitamin A. It is highly unfortunate, however, that the method of synthesis of this vitamin A has not been revealed, although the authors stated that it differs from any methods heretofore described in the literature.

Since the correction of the page proof, Isler, Huber *et al.* (1947) published an important article on the synthesis of vitamin A. They used a slight modification of the methods already published by Milas (1945a,e). The new modification was also developed in this Laboratory by Milas, Grossi, and Harrington (1947) and complete data will be published elsewhere.

REFERENCES

1. Arens, J. F., and van Dorp, D. A. 1946. *Nature* **157,** 190.
2. Bogert, M. T. 1938. Organic Chemistry, edited by H. GILMAN, Vol. 2, p. 1138. Wiley, New York.
3. Campbell, K. N., and Campbell, B. K. 1942. *Chem. Revs.* **31,** 77.
4. Campbell, K. N., and Edy, L. T. 1941. *J. Am. Chem. Soc.* **63,** 216, 2683.
5. Coulson, C. A. 1939. *Proc. Roy. Soc. London* **169A,** 413.
6. Cristol, S. J. 1947. *J. Am. Chem. Soc.* **69,** 338.
7. Cymerman, J., Heilbron, I. M., Jones, E. R. H., and Lacey, R. N. 1946. *J. Chem. Soc.* 727.
8. Davies, W. H., Heilbron, I. M., Jones, W. E., and Lowe, A. 1935. *J. Chem. Soc.* 584.
9. Deuel, H. J., Jr., Meserve, E. R., Sandoval, A., and Zechmeister, L. 1946. *Arch. Biochem.* **10,** 491.
10. van Dorp, D. A. and Arens, J. F., with van Dijk, G. M., Favier, J., Koster, P., and de Rover, H. 1946. *Rec. trav. chim.* **65,** 338.
11. Eekelen, M. van, Emmerle, A., Julius, H. W., and Wolff, L. K. 1933. *Nature* **132,** 171.
12. Embree, N. D., and Shantz, E. M. 1943. *J. Am. Chem. Soc.* **65,** 906.
13. Euler, H. von, Karrer, P., and Zubris, A. 1934. *Helv. Chem. Acta* **17,** 24.
14. Fuson, R. C., Arnold, R. T. and Cooke, H. G., Jr. 1938. *J. Am. Chem. Soc.* **60,** 2272.
15. Fuson, R. C., and Christ, R. E. 1936. *Science* **84,** 294.
16. Gilman, H., and van Ess, P. R. 1933. *J. Am. Chem. Soc.* **55,** 1258.
17. Gould, R. G., and Thompson, A. F., Jr. 1935. *J. Am. Chem. Soc.* **57,** 340.
18. Hanze, A. R., Conger, T. W., Wise, E. C., and Weisblat, D. I. 1946. *J. Am. Chem. Soc.* **68,** 1389.
19. Hawkins, E. G. E., and Hunter, R. F. 1944. *Biochem. J.* **38,** 34.
20. Heilbron, I. M., Heslop, R. N., Morton, R. A., Webster, E. T., Rea, J. L., and Drummond, J. C. 1932. *Biochem. J.* **26,** 1178.
21. Heilbron, I. M., Johnson, A. W., Jones, E. R. H., and Spinks, A. 1942. *J. Chem. Soc.* 727.
22. Heilbron, I. M., and Jones, W. E. 1936. *Chemistry & Industry* **55,** 813.
23. Heilbron, I. M., Jones, W. E., and Bacharach, A. L. 1944. *Vitamins & Hormones* **2,** 155.
24. Heilbron, I. M., Jones, W. E., Lowe, A., and Wright, H. R. 1936. *J. Chem. Soc.* 561.
25. Heilbron, I. M., Jones, E. R. H., and O'Sullivan, D. G. 1946a. *Nature* **157,** 485.
26. Heilbron, I. M., Jones, E. R. H., and O'Sullivan, D. G. 1946b. *J. Chem. Soc.* 866.
27. Heilbron, I. M., Morton, R. A., and Webster, E. T. 1932. *Biochem. J.* **26,** 1194.
28. Huckel, W., Tappe, W., and Legutke, G. 1940. *Ann.* **543,** 191.
29. Hunter, R. F., and Williams, N. E. 1945. *J. Chem. Soc.* 554.
30. Ishikawa, S., and Matsuura, T. 1937. *Sci. Repts. Tokyo Bunrika Dargaku* **3A,** 173.
31. Isler, O., Huber, W., Ronco, A., and Kofler, M. 1946. Jubilee Volume to Emil C. Barrell, p. 31. Hoffman-LaRoche and Co., Basle.
32. Isler, O., Kofler, M., Huber, W., and Ronco, A. 1946. *Experientia* **2,** 31.

33. Karrer, P., 1945. *Helv. Chim. Acta* **28,** 474.
34. Karrer, P., and Jucker, E. 1945a. *Helv. Chim. Acta* **28,** 300.
35. Karrer, P., and Jucker, E. 1945b. *Helv. Chim. Acta* **28,** 471.
36. Karrer, P., and Jucker E. 1945c. *Helv. Chim. Acta* **28,** 427.
37. Karrer, P., and Jucker, E. 1945d. *Helv. Chim. Acta* **28,** 717.
38. Karrer, P., Jucker, E., and Schick, E. 1946. *Helv. Chim. Acta* **29,** 704.
39. Karrer, P., and Morf, R. 1933. *Helv. Chim. Acta* **16,** 625.
40. Karrer, P., Morf, R., and Schöpp, K. 1931. *Helv. Chim. Acta* **14,** 1036, 1431.
41. Karrer, P., Salomon, H., Morf, R., and Walker, O. 1932. *Helv. Chim. Acta* **15,** 878.
42. Karrer, P., and Rüegger, A. 1940. *Helv. Chim. Acta* **23,** 284.
43. Karrer, P., and Rüegger, A. 1944. *Helv. Chim. Acta* **27,** 1588.
44. Karrer, P., and Rutschmann, J. 1944. *Helv. Chim. Acta* **27,** 284.
45. Kipping, F. B., and Wild, F. 1939. *Chemistry & Industry* **58,** 802.
46. Koch, H. P. 1942. *Chemistry & Industry* 273.
47. Köster, H. 1944. *Ber.* **77B,** 553.
48. Krauze, M. V., and Slobodin, Y. M. 1940. *J. Gen. Chem. U.S.S.R* .**10,** 907; *Chem. Abstracts* 1941. **35,** 3237.
49. Kuhn, R., and Morris, C. J. O. R. 1937. *Ber.* **70,** 853.
50. Kuhn, R., and Morris, C. J. O. R. 1940. G. Pat. 696, 084, Aug. 15.
51. Kuhn, R., and Morris, C. J. O. R. 1941a. U. S. Pat. 2, 233, 375, Feb. 25.
52. Kuhn, R., and Morris, C. J. O. R. 1941b. U. S. Pat. 2, 239,491, April 22.
53. Kuhn, R., and Wallenfels, K. 1938. *Ber.* **71B,** 783.
54. Lund, H. 1937. *Ber.* **70,** 1520.
55. Milas, N. A. 1939. U. S. Pat. 2, 173,629, Sept. 19.
56. Milas, N. A. 1945a. U. S. Pat. 2,369,156, Feb. 13.
57. Milas, N. A. 1945b. U. S. Pat. 2,369,157, Feb. 13.
58. Milas, N. A. 1945c. U. S. Pat. 2,369,158, Feb. 13.
59. Milas, N. A. 1945d. U. S. Pat. 2,369,159, Feb. 13.
60. Milas, N. A. 1945e. U. S. Pat. 2,369,160, Feb. 13.
61. Milas, N. A. 1945f. U. S. Pat. 2,369,161, Feb. 13.
62. Milas, N. A. 1945g. U. S. Pat. 2,369,162, Feb. 13.
63. Milas, N. A. 1945h. U. S. Pat. 2,369,163, Feb. 13.
64. Milas, N. A. 1945i. U. S. Pat. 2,369,164, Feb. 13.
65. Milas, N. A. 1945j. U. S. Pat. 2,369,165, Feb. 13.
66. Milas, N. A. 1945k. U. S. Pat. 2,369,166, Feb. 13.
67. Milas, N. A. 1945l. U. S. Pat. 2,369, 167, Feb. 13.
68. Milas, N. A. 1945m. U. S. Pat. 2,369,168, Feb. 13.
69. Milas, N. A. 1945n. U. S. Pat. 2,382,085, Aug. 14.
70. Milas, N. A. 1945o. U. S. Pat. 2, 382,086, Aug. 14.
71. Milas, N. A. 1946. *Science* **103,** 58.
72. Milas, N. A. 1947. U. S. Pat. 2,415,834, Feb. 18.
73. Milas, N. A., and Edgerton, R. O. 1947. Unpublished results.
74. Milas, N. A., Edgerton, R. O., Sakal, E., and Gladding, J. K. 1947. Unpublished results.
75. Milas, N. A., Heggie, R. S., and Mason, H. S. 1947. Unpublished results.
76. Milas, N. A., and Kovitz, J. 1947. Unpublished results.
77. Milas, N. A., Lee, S. W., Sakal, E., Wohlers, H. C., McDonald, N. S., Grossi, F. X., and Wright, H. F. 1947. Submitted for publication.
78. Milas, N. A., Lee, S. W., Schuerch, Jr., C., Edgerton, R. O., Plati, J. T., Grossi, F. X., Weiss, Z., and Campbell, M. A. 1947. Submitted for publication.

79. Milas, N. A., and McAlevy, A. J. 1935. *J. Am. Chem. Soc.* **57,** 580.
80. Milas, N. A. and Sakal, E. 1947. Unpublished results.
81. Milas, N. A., Sakal, E., Grossi, F. X. and Pitt, R. B. 1947. In preparation.
82. Milas, N. A., Sakal, E., Plati, J. T., Rivers, J. T., Gladding, J. K., Grossi, F. X., Weiss, Z., Pitt, R. B., Campbell, M. A., and Wright, H. F. 1947. Submitted for publication.
83. Milas, N. A., Sakal, E., Plati, J. T., and Yamins, J. L. 1947. In preparation.
84. Morrell, R. S., Bolan, T. R., Davis, R. R., Marks, S., Phillips, E. O., and Sim, W. S. 1942. *Trans. Faraday Soc.* **38,** 1627.
85. Oroshnik, W. 1945. *J. Am. Chem. Soc.* **67,** 1627.
86. Pauling, L. 1939. *Fortschr. Chem. Organ. Naturstoffe* **3,** 203.
87. Private communication from Merck and Co. through the Research Corp. of N. Y.
88. Rivers, J. T., Jr. 1941. Ph. D. Thesis, Mass. Inst. Tech.
89. Rivers, J. T., Jr., and Milas, N. A. 1947. Unpublished results.
90. Robeson, C. D., and Baxter, J. G. 1945. *Nature* **155,** 300.
91. Robeson, C. D., and Baxter, J. G. 1947. *J. Am. Chem. Soc.* **69,** 136.
92. Shantz, E. M. 1946. *J. Am. Chem. Soc.* **68,** 2553.
93. Shantz, E. M., Cawley, J. D., and Embree, N. D. 1943. *J. Am. Chem. Soc.* **65,** 904.
94. Sobotka, H., and Bloch, E. 1944. *Chem. Revs.* **34,** 435.
95. Sobotka, H., Bloch, E., and Glick, D. 1943. *J. Am. Chem. Soc.* **65,** 1961.
96. Swern, D. E. 1947. *J. Am. Chem. Soc.* **69,** 1692.
97. Winstein, S., Pressman, D., and Young, W. G. 1939. *J. Am. Chem. Soc.* **61,** 1645.
98. Wittig, A., and Fartmann, B. 1943. *Ann.* **554,** 213.
99. Wittig, A., and Wiemer, W. 1930. *Ann.* **483,** 144.
100. Young, W. G., Cristol, S. J. and Skei, T. 1943. *J. Am. Chem. Soc.* **65,** 2099.
101. Zechmeister, L. 1944. *Chem. Revs.* **34,** 267.
102. Zechmeister, L., LeRosen, A. L., Went, F. W., and Pauling, L. 1941. *Proc. Natl. Acad. Sci. U. S.* **27,** 468.
103. Ziegler, K. 1947. German Pat. Application, as mentioned in *Rec. Trav. chim.* **65,** 338.
104. Ziegler, K., Spath, A., Schaaf, E., Schumann, W., and Winkelmann, E. 1942. *Ann.* **551,** 80.
105. van Dorp, D. A., and Arens, J. F. 1947. *Nature* **160,** 189.
106. Hawkins, E. G. E., and Hunter, R. F. 1944. *J. Chem. Soc.* 411.
107. Milas, N. A., and Harrington, T. M. 1947. *J. Am. Chem. Soc.* **69,** 2247 .
108. Batty, J. W., Buraway, A., Harper, S. H., Heilbron, I. M., and Jones, W. E. 1938. *J. Chem. Soc.* 175.
109. Finholt, A. E., Bond, A. C., Jr., and Schlessinger, H. I. 1947. *J. Am. Chem. Soc.* **69,** 1199.
110. Cawley, J. D., Robeson, C. D., Weisler, L., Shantz, E. M., Embree, N. D., and Baxter, J. G. 1947. Paper presented before the American Chemical Society Meeting (September), New York City.
111. Isler, O., Huber, W., Ronco, A., and Kofler, M. 1947. *Helv. Chim. Acta* **30,** 1911.
112. Milas N. A., Grossi, F. X., and Harrington, T. M. 1947. Unpublished results.

Physiological Availability of the Vitamins

By DANIEL MELNICK AND BERNARD L. OSER

CONTENTS

Page
I. Introduction . . . 39
II. Limitations in the Non-Biological Methods for Determining the Vitamins . 41
  1. Physical Methods . . . 41
  2. Chemical Methods . . . 42
  3. Microbiological Methods . . . 44
III. Measurement of Physiological Availability . . . 47
  1. Animal Tests . . . 48
  2. Human Assay Procedures . . . 49
IV. Factors Influencing the Physiological Availability of the Vitamins 57
  1. Fat-Soluble Vitamins . . . 57
    a. Vitamin A . . . 58
    b. Vitamin D . . . 60
    c. Vitamin E . . . 60
    d. Vitamin K . . . 61
  2. Water-Soluble Vitamins . . . 61
    a. Ascorbic Acid . . . 62
    b. Thiamine . . . 64
    c. Riboflavin . . . 68
    d. Niacin . . . 69
    e. Pantothenic Acid . . . 72
    f. Vitamin B_6 . . . 72
    g. Biotin . . . 73
    h. *p*-Aminobenzoic Acid . . . 75
    i. Folic Acid . . . 75
    j. Inositol . . . 77
    k. Choline . . . 78
V. Unsolved Problems of Physiological Availability in Human Nutrition . . . 79
  1. Conditioned Malnutrition . . . 79
  2. Biosynthesis of Vitamins . . . 80
  3. Vitamin-Vitamin and Vitamin-Amino Acid Interrelationships . . . 81
  4. Antivitamins . . . 82
  5. Availability of the Water-Insoluble Forms of the Water-Soluble Vitamins . . . 82
  6. Availability of the Niacin Derivative in Cereal Products 83
  7. Influence of Treating Foods with Various Salts During Processing . . 84
VI. Concluding Remarks . . . 84
  References . . . 85

I. INTRODUCTION

Among the urgent needs for further research reported at the National Nutrition Conference in 1941 was "Improvement of presently known chemical and biological procedures for estimating the amounts of the essential nutrients in foods and their physiological availability." In this statement are recognized not only the advantages of chemical and microbiological assays for vitamins, a field of methodology in which outstanding progress has been achieved, but also the basic principle that content and availability are not synonymous terms in the case of the vitamins any more than they are with respect to minerals, proteins, fats or carbohydrates. The studies by Horwitt, Cowgill and Mendel (1936a, 1936b) deal specifically with the latter dietary factors. They point out the errors inherent in determining the protein quality of a food simply by multiplying its nitrogen content by a factor; available fat by the percentage of ether extract; crude fiber as the organic material insoluble in boiling acid and alkali; available carbohydrate by difference; or the mineral nutrients by analysis of ashed foods for calcium, phosphorus and iron.

Despite the commendable efforts of food analysts to substitute more reliable analytical technics for determining the content of available nutrients, these workers have persisted in using the older technics even in the analysis of plant products, which are even more likely than animal foods to yield erroneous values from the standpoint of nutrient contributions. The inertia in adopting the newer, but more complex, procedures is illustrated in the recent publication prepared by the Bureau of Human Nutrition and Home Economics of the United States Department of Agriculture in cooperation with the National Research Council (1945) which, perforce, cites the analytical data going back more than 50 years. The need for further study along the lines of physiological availability has recently been emphasized by Hart (1945).

Analytical methods are usually directed toward establishing the total quantity of a specific nutrient present in a food. On the other hand, that fraction of the total nutrient which is absorbed and utilized is measured by biological tests, preferably on the animal species for which the food is intended. Total nutrient content should be employed by the nutritionist only as the denominator in a fraction expressing the percentage availability of the nutrient under a given set of circumstances.

Vitamins may exist in nature in such firm union with proteins or other compounds that either they fail to respond to the characteristic reactions by which they may be determined chemically or they are not utilized by the test microorganism unless released by preliminary hydrolysis. The necessary pretreatment of the test sample may not be duplicated by the digestive process during the gastrointestinal journey. On the other hand, various enzyme systems within the animal organism cannot be exactly simulated *in vitro*. Thus, analytical values for vitamin content may be considerably lower than those obtained by biological assay; this has been particularly true in the determination of folic acid content. Furthermore, vitamin-vitamin interrelationships (Moore,

1945) and vitamin-amino acid interrelationships (Mitchell, 1943) can either increase or decrease the biological response to a given dietary. Intestinal synthesis or destruction of the vitamins (Najjar and Barrett, 1945), dependent on the composition of the food consumed, and the concomitant ingestion of anti-vitamins (Woolley, 1946a) can greatly modify the available vitamin content of a mixed ration.

In this review on physiological availability of the vitamins, the discussion will not be restricted simply to degree of vitamin absorption from the gastrointestinal tract, but will also include mention of other factors which modify (enhance or diminish) the biological response following ingestion of the vitamins in question. Rather than review these other phases of vitamin metabolism, which has been done in some of the papers referred to above, one or two reports will be cited to illustrate each point, since it is not possible, within the space limitations of this review, to discuss all papers bearing on this subject. The influence of each of the related factors mentioned above on physiological availability will be discussed under the sections dealing with the individual vitamins.

II. LIMITATIONS IN THE NON-BIOLOGICAL METHODS FOR DETERMINING THE VITAMINS

Prior to a biological assay (animal or human) for measuring the physiological availability of a vitamin, it is desirable, and in some cases necessary, that the total intake be estimated by a reliable procedure. Often technics are chosen because of their relative simplicity or because they yield reproducible values. Reproducibility as an index of reliability is frequently confused with specificity. In this section of the review, it is not our intention to discuss fully the limitations of the physical, chemical and microbiological methods for estimating the vitamins. Typical examples will be presented and these should suffice for the discussion.

1. *Physical Methods*

The light-absorbing or fluorescent properties of certain vitamins are frequently employed as the basis for physical methods of estimation. The color of the vitamin may be visible, as in the case of carotene (A.O.A.C., 1945), or it may be invisible as in the case of preformed vitamin A. Vitamin A absorbs ultraviolet radiation (Oser *et al.*, 1943a) maximally at 325–328 mμ; riboflavin is yellow and may be measured (U.S.P., 1942) colorimetrically or photometrically (maximum 450 mμ). Pyridoxine is colorless and may be measured by its absorption in the ultraviolet (Melnick *et al.*, 1945). If the system being examined is sufficiently pure, it becomes a simple matter to determine the absorption at a specific wavelength and from that to calculate the vitamin content of the test material. The fluorescence of riboflavin (Hodson and Norris, 1939; Scott *et al.*, 1946) and of vitamin A ester (Sobotka *et al.*, 1943) have been employed in similar fashion for the estimation of these factors.

The non-critical use of such physical methods, however, has frequently led to erroneous values in determining the vitamin content of foods and pharmaceuticals as the following examples will illustrate. The carotenoid pigments vary widely in biological activity. The compounds can exist in a large number of stereoisomeric forms due to *cis-trans* configuration about the conjugated bonds (Polgár and Zechmeister, 1942, 1943, 1944, 1945; Zechmeister *et al.*, 1943). β-Carotene alone has 20 stable isomers. These are differentiated by the heights and wavelengths of the maxima in the visible and ultraviolet absorption spectra and by chromatographic separation. About half of the isomers of β-carotene have been found in nature. The available evidence (Deuel *et al.*, 1945) indicates that differences exist in their provitamin A activity. In the AOAC procedure for determining carotene, methods are presented which involve chromatographic separation of the carotenoid pigments and identification of the carotene by its specific absorption at the more important wavelengths of its spectrum. However, these procedures are quite involved and the tendency in practice is to confine the analysis to a determination of the crude carotene content.

In the spectrophotometric analysis of vitamin A, the effect of irrelevant absorption may often be reduced by conducting the tests on unsaponifiable extracts (Oser *et al.*, 1943a). However, even this procedure may fail, as indicated by atypical ultraviolet absorption curves of unsaponifiable extracts of foods fortified with pure vitamin A concentrates (Oser *et al.*, 1943b). Before the spectrophotometric method can be used in the assay of such materials, it becomes necessary to subtract irrelevant absorption from the total absorption of the vitamin-fortified product or specifically to remove the absorption due to vitamin A, for example, by destructive ultraviolet irradiation, thereby obtaining a blank correction. In either case, the method loses precision because of the very high blank values. Control tests are also required to demonstrate that irrelevant absorbing substances may not have been created during the irradiation process. Likewise, the non-critical use of fluorometric methods for the estimation of the vitamins may yield erroneous values due to a tendency on the part of analysts to modify technics which the original authors have proposed as essential for the specificity of the methods.

2. Chemical Methods

Chemical assays are based upon more or less specific reactions which may be colorimetric, fluorometric or titrimetric. A tendency to favor the reproducible simpler technics in preference to more involved but specific methods for estimating vitamin content of foods is apparent in reviewing the literature of even the past few years.

The antimony trichloride procedure (Oser *et al.*, 1943b) and the glycerol dichlorohydrin (Sobel and Werbin, 1945) method are suitable for the estimation of preformed vitamin A provided the test system contains negligible quantities of carotenoids and sterols. Brew and Scott (1946) have emphasized the limitations of existing chemical procedures for the determination of vitamin A in low potency materials (*e.g.*, mixed feeds) and made a notable contribution to the solution of the problem by using preliminary chromatographic adsorption technics and introducing a system of correction dependent upon the effect of light on the kinetics of the antimony trichloride reaction. This paper should stimulate further studies toward solution of a difficult but important problem.

In the field of vitamin C methodology the titrimetric procedure (Bessey and King, 1933; Musulin and King, 1936; A.O.A.C., 1945) has been used indiscriminately for assaying all types of food products. This method does yield reliable data in the case of certain fresh fruits and their juices. However, when assays are made on dehydrated vegetables and other processed foods involving the use of heat, compounds are formed that are capable of reducing the dye 2,6-dichlorophenolindophenol. Under such circumstances more reliable estimates of ascorbic acid content may be obtained by taking into consideration the rate at which ascorbic acid reduces the dye (Hochberg *et al.*, 1943a), the different rates of combination of ascorbic acid, and other indophenol-reducing substances, with formaldehyde at specific pH values to yield complexes which then react differently (Lugg, 1942), using a technic dependent upon the selective solubility in xylene of the non-reduced 2,6-dichlorophenolindophenol in acid solution (Pepkowitz, 1943; Robinson and Stotz, 1945), or using the method based on the reaction of dehydroascorbic acid with 2,4-dinitrophenylhydrazine to form an osazone (Roe and Kuether, 1943). Even these methods tend to overestimate the true vitamin C content (Hochberg *et al.*, 1943b) in some heat-processed foods, particularly stored dehydrated foods. The importance of analyzing raw and cooked foods for dehydroascorbic acid (the partially oxidized form of the vitamin which is still biologically active) has been repeatedly emphasized (Hochberg *et al.*, 1943a; McMillan and Todhunter, 1946; Smith and Caldwell, 1945), but too frequently neglected in practice. It would seem that the method of Stewart and Sharp (1945) for the determination of vitamin C in the presence of interfering reducing substances should merit more attention by those interested in vitamin C methodology. In this procedure, ascorbic acid and interfering substances are catalytically oxidized by the addition of ascorbic acid oxidase. Indophenol dye titrations are then conducted on the solutions before and after specific reduction of the dehydroascorbic acid to ascorbic acid by a suspension of *Escherichia coli* or *Staphylococcus albus*. It is unfortunate that food analysts sometimes aim toward the completion of the greatest number of assays within a given time rather than toward improving the reliability of the results obtained.

The vitamin A paper by Brew and Scott (1946), and that on ascorbic acid by Stewart and Sharp (1945), are mentioned as illustrative of constructive papers in the field of vitamin methodology where the emphasis has been placed on reliability of the assay methods and not primarily on reproducibility and simplicity.

In the same sense the papers by Ewing and collaborators (Ewing *et al.*, 1943), Sobel and associates (1945), and DeWitt and Sullivan (1946), on the chemical determination of vitamin D may be cited. Even in these cases, however, the chemical procedures cannot be employed for differentiating vitamin D_2 from vitamin D_3 and species differences in the ability to utilize these two forms of the vitamin are known to exist. Likewise, most chemical methods fail to differentiate the various forms of tocopherol, which vary widely in biological potency (Joffe and Harris, 1943). Furthermore, the naturally occurring tocopherols are far more potent, mole for mole, than the synthetic products (Harris *et al.*, 1944). It is pertinent that Hickman and Harris (1946) regard as the most critical and important problem in vitamin E research the development of reliable specific methods for the determination of α-, β-, and γ-tocopherols in foods and tissues. Some success in this field has already been claimed (Baxter *et al.*, 1943; Quaife, 1944; Fischer, 1945). Critical discussions have likewise been reported bearing on the reliability of the chemical procedures for niacin (Melnick, 1942) and for vitamin B_6 (Melnick *et al.*, 1945).

3. Microbiological Methods

The workers in the field of animal assay have frequently felt that they have the last word insofar as the criterion of specificity is concerned. This feeling of security can now be seen among those who prefer the microbiological assay method to the exclusion of all other analytical procedures. The emphasis on the part of the worker using microbiological methods has been directed toward the development of extraction and hydrolytic procedures which tend to yield maximal figures for the material being assayed. In Table I are listed typical technics employed in the determination of the vitamins of the B complex by microbiological assay. It is quite apparent that most of the extraction and hydrolytic procedures have no exact counterpart within the animal organism itself. The question may then be asked—is the microbiologist measuring total rather than available vitamin content?

TABLE I

Preparation of Samples for Microbiological Assays

Vitamin	Extraction and hydrolytic procedure	Test microorganism	Reference
Thiamine	Heat 20 min., 100°C., pH 3.5	*Saccharomyces cerevisiae*	235
Riboflavin	Autoclave 30 min., 15 lb., 0.1 *N* HCl	*Lactobacillus casei*	275, 8
Niacin	Autoclave 30 min., 15 lb., 1.0 *N* H_2SO_4	*Lactobacillus arabinosus*	275, 8
Pantothenic acid	Digest with clarase, heat 20 min., 100°C. at pH 7	*Lactobacillus arabinosus*	11, 248
Vitamin B_6	Autoclave 60 min., 20 lb., 0.055 *N* or 2 *N* H_2SO_4	*Saccharomyces carlsbergensis*	10, 156
Biotin	Autoclave 60 min., 15 lb., 6 *N* H_2SO_4	*Lactobacillus arabinosus*	291
Pteroylglutamic acid (Folic acid).	Digest by specific enzyme from chicken pancreas or hog kidney	*Lactobacillus casei* or *Streptococcus faecalis*	30, 23
p-Aminobenzoic acid.	Autoclave 30 min., 13.5 lb., 1 *N* NaOH	*Acetobacter suboxydans*	226
Choline	Autoclave 120 min., 15 lb., 1 *N* H_2SO_4	Cholineless mutant of *Neurospora crassa*	107
Inositol	Reflux 6 hr., 7 *N* HCl	*Saccharomyces cerevisiae*	122

Caution should also be exercised before accepting the microbiological assay as inherently specific for the vitamin in question. For example, pteroylglutamic acid (folic acid) seems to be required by bacteria for the synthesis of the pyrimidine, thymine, so that, in the presence of excessive quantities of the latter, a deficiency of folic acid in the medium is no longer a limiting factor (Stokes, 1944). It has been found that in the microbiological methods for riboflavin (Bauernfeind *et al.*, 1942; Strong and Carpenter, 1942), pantothenic acid (Bauernfeind *et al.*, 1942; Neal and Strong, 1943), and biotin (Williams and Vieger, 1945), fat has a stimulatory effect upon microbial growth. Although the fat itself fails to react like the vitamin in the assay, the presence of the fat is responsible for greatly augmenting the microbiological response. Starch likewise has a stimulatory effect on *Lactobacillus casei* in the presence of critical amounts of riboflavin (Scott *et al.*, 1941; Andrews *et al.*, 1942). Furthermore, glucose, if present in variable amounts in the unknown material, may be responsible for erroneous results. During the metabolism of the microorganism greater acid production occurs in the presence of more glucose (Light. and Clarke, 1943; Stokes and Martin, 1943). Buffers permit a greater conversion of glucose to lactic acid to occur, since the low pH inhibits bacterial growth (Stokes and Martin, 1943). These variable factors in microbiological assays are gradually becoming recognized and controlled. Thus, in the case of riboflavin, pantothenic acid and biotin assays, preliminary ether extraction or simply filtration through paper is carried out for all test materials or extracts in order to eliminate the fat. There is a tendency to increase the glucose content of a basal medium and to increase the buffering capacity to an optimum determined by the salt concentration (Light and Clarke, 1943; Stokes and Martin, 1943).

A serious limitation in microbiological assays is that these procedures may measure related compounds with similar apparent activity for the microorganism but not for the higher organism. Thus, in the case of thiamine, microorganisms are frequently employed which respond simply to the pyrimidine or thiazole portion of the molecule (Robbins, 1938). Some require both, but not necessarily in combination as found in thiamine (Robbins and Kavanagh, 1938a, 1938b). Only intact thiamine is biologically active for the animal organism. It is also known that, during the decomposition of thiamine, the pyrimidine moiety is frequently left unaltered. Thus, erroneously high results may be obtained in the assay of various test materials by the use of such microbiological procedures unless this type of interference is recognized and steps taken to correct for it. By the incorporation of a sulfite blank, it is possible to eliminate the stimulation due to thiamine alone and by difference obtain an estimation of the true vitamin B_1 content (Schultz *et al.*, 1942). Some organisms used for the estimation of biotin can also use pimelic acid (du Vigneaud *et al.*, 1942) as a precursor for the synthesis of this vitamin. Pantothenic acid is made up of lactone and β-alanine moieties. Some organisms require the lactone, others the β-alanine (Woolley, 1939). However, the rat requires both fractions combined in the form of pantothenic acid for it to be effective (Oleson *et al.*, 1939). Large amounts of alanine can completely replace pyridoxine (and presumably pyridoxamine) as a growth factor for *Streptococcus fecalis* (Snell and Guirard, 1943). Only by the use of proper test microorganisms are specific analyses for the vitamins possible.

In some cases a fortunate circumstance is found where related chemical compounds which possess vitamin activity for the animal organism exhibit microbiological activity for the microorganism of a comparable degree. Thus, when the yeast, *Saccharomyces carlsbergensis*, is employed for the determination of vitamin B_6 activity (Atkin *et al.*, 1943), the estimates include the quantities of pyridoxine, pyridoxal, pyridoxamine (Snell and Rannefeld, 1945), which may be present. The responses to the other members of the vitamin B_6 group are equivalent to those obtained with pyridoxine and similar findings are noted in animal assays (Snell and Rannefeld, 1945; Melnick *et al.*, 1945; Sarma *et al.*, 1946).

Finally, microorganisms may fail to measure vitamin derivatives which nevertheless are active for the animal organism and may therefore be responsible for erroneously low estimates. In the case of pteroylglutamic (folic) acid the microbiological procedures appear to measure only a small fraction of this vitamin in some foods. Technics have been proposed (Burkholder *et al.*, 1945; Bird *et al.*, 1946) for the enzymic conversion of the conjugated forms of this vitamin to the free vitamin. In some foods, notably yeast, the conjugated vitamin may constitute by far the major portion of the nutrient present (Burkholder *et al.*, 1945). Evidence for the conversion of xanthopterin (chemically related to the pterin group in folic acid) by an enzyme system in fresh liver into a substance which stimulates the growth of *Streptococcus fecalis* has also been reported (Wright *et al.*, 1945). Even when the *in vitro* enzymic technics (Burkholder *et al.*, 1945; Bird *et al.*, 1946) are employed to render the conjugates of pteroylglutamic acid available to the microorganisms, the biological responses in the animal organism may still be significantly greater. This has been shown to be due to the presence in food materials of inhibitors for the enzyme system which are rendered inactive in the animal organism (Bird *et al.*, 1946).

In this same category of microbiological availability may be mentioned the non-utilization by the microorganism of the niacin precursor in cereal products unless the vitamin derivative in such products is first subjected to acid or alkaline hydrolysis (Melnick, 1942; Krehl and Strong, 1944; Krehl *et al.*, 1944).

It is apparent from the foregoing discussion of the microbiological procedures that, when the proper microorganism is selected and the necessary precautions taken, these methods may be used for the determination of total vitamin content of a test system. How closely the values obtained reflect the physiologically available vitamin content of foods has yet to be ascertained.

III. MEASUREMENT OF PHYSIOLOGICAL AVAILABILITY

The limitations of physical, chemical, and microbiological procedures for the determination of the available vitamin content of foods have been discussed. Bioassays with the animal organism, and preferably with the species of interest, must be regarded as the only basis of reference for estimating the availability of vitamins. In the present review partiality will be shown to the human bioassay technic, since one of the primary objectives in animal research is the discovery of factors and mechanisms directly applicable to human nutrition.

1. Animal Assay Procedures

Animal assays are usually dependent upon the measurement of two types of responses, *viz.*, either the overall growth response, or a specific physiologic response, such as calcification induced by vitamin D or the cure of polyneuritis by thiamine. In the determination of vitamin A or C, the growth method is usually employed. The general procedure of bioassay is to place the animals on a diet containing all factors essential for the normal growth of the organism, with the exception of the vitamin under assay. When a plateau or decline in weight of the animal is noted, the test substance is fed in addition to the basal ration. Increase in weight during a limited period is compared with the weight increase in the control group of animals fed a known amount of the vitamin. The details of such methods may be found in official compendia (A.O.A.C., 1945; U.S.P., 1943). For purposes of quantitative evaluation of biological vitamin potency, the graded response method must be used. This involves feeding each vitamin supplement to two or more groups of animals at selected, graded dose levels so as to establish the relation between dose and response as expressed on some quantitative scale. The values obtained, be they weight gains or the rating of a response such as degree of calcification, are plotted against the doses, or, preferably, the logarithm of the doses, since this transformation is more likely to yield a linear relationship between dose and effect. The simplest method for calculating potency involves interpolating responses obtained for the unknown on the reference curve and averaging "found" values for all assay groups. By this procedure the slope of the response curve is based on the use of only half the data obtained. However, the method of interpretation based on the analysis of variance enables one to obtain an estimate of potency of the unknown, together with a statistical index of the reliability of the estimate using all the data of the assay. It is possible that the slope of response of the unknown material may not be the same as that for the reference material. That is, the increment in growth or in per cent of bone ash with increasing dosage of the unknown may not be the same as that for the reference material. Because the assumed potency, and hence the dosage levels, may be too far from the actual potency, the responses obtained may not fall within the critical span of the response curve. This is especially likely to occur, and may escape detection, in minimal potency assays. The significance of these variables, and whether or not they are sufficient to vitiate a given assay, can be determined by properly planned and statistically interpreted multiple level biological assays (Bliss and Marks, 1939).

As in the case of the microbiological assay procedures, the response obtained during animal assays may be the resultant of many factors. Two products may contain the same quantity of a given vitamin, but vary widely in biological potency. It is precisely this deviation from vitamin content with which we are concerned in the present discussion. As long as conclusions are restricted to the species employed for the biological assay, the estimates can be regarded as indicative of available vitamin content. Generalizations in the field of nutrition, based upon the results obtained with one species, are apt to prove unwarranted. Indeed, it was by studies on species other than the rat, *viz.*, the mouse, chick, guinea pig, dog and monkey, that many new dietary factors have been discovered.

The animal assays are usually regarded as measuring physiologically available nutrients, but they are not always reliable when applied to human nutrition. Among the factors affecting animal assays are the pathologically depleted state of the experimental animal, the use of purified or unbalanced basal rations whose composition may be specifically related to the utilization of the factor under test, and the need for subdivision or extraction of the test materials. These depart considerably from the conditions underlying the condition of use in human nutrition, and therefore warrant the exercise of caution in the application of laboratory data.

2. Human Assay Procedures

In the recent literature, many reports have appeared attempting to evaluate the physiological availability of the vitamins, using man as the test animal (Hawley *et al.*, 1936; Clayton and Folsom, 1940; Todhunter and Fatzer, 1940; Cahill, 1941; Melnick, Robinson and Field, 1941; Parsons and Collord, 1942; Parsons *et al.*, 1942; Clayton and Borden, 1943; Meyer and Hathaway, 1944; Reinhold *et. al.*, 1944; Hartzler, 1945; Hochberg *et al.*, 1945b; 1945c; 1945d; Melnick *et al.*, 1945a; 1945b; 1945c; Oser *et al.*, 1945; Parsons *et al.*, 1945b; Williamson, and Parsons, 1945; Bethell *et al.*, 1946; Furter *et al.*, 1946; Ness *et al.*, 1946; Welch *et al.*, 1946). These studies have been concerned only with the water-soluble vitamins, since the basis of the assay technic is the measurement of the urinary excretion of these factors as such or as derivatives. The fat-soluble vitamins are not normally excreted in the urine. For this reason, human availability studies of the fat-soluble vitamins have depended upon the measurement of increase of the blood level of the fat-soluble vitamins following dosage (Curtis and Kline, 1939; Adlersberg and Sobotka, 1943; Clausen *et al.*, 1946; Sobel *et al.*, 1946). Attempts to measure the unabsorbed fat-soluble vitamins by analysis of the feces have also been made (Fraps, 1946).

The general plan in human physiological availability studies of the water-soluble vitamins is to compare the urinary excretion of the given factor when taken as part of the test dietary with the urinary excretion when the same quantity of vitamin is taken in pure aqueous solution, the form in which it is postulated to be most readily available for absorption. Extra quantities of the vitamin or derivative appear in the urine following dosage with the test material only after these nutrients have been extracted and absorbed from the gastrointestinal tract.

In some studies blood analyses are conducted simultaneously. However, these have only qualitative value, since a small increment in blood value can accompany a very large increase in the excretion of the water-soluble vitamins in the urine. Investigations with normal subjects before and after profuse sweating have indicated that the losses of the water-soluble vitamins in sweat are negligible. These studies have been concerned with loss of ascorbic acid (Kirch *et al.*, 1943; Mickelsen and Keys, 1943; Tennet and Silber, 1943; Sargent *et al.*, 1944; Shields *et al.*, 1945), thiamine (Cornbleet *et al.*, 1943; Mickelsen and Keys, 1943; Tennet and Silber, 1943), riboflavin (Cornbleet *et al.*, 1943; Mickelsen and Keys, 1943; Tennet and Silber, 1943), niacin (Cornbleet *et al.*, 1943; Sargent *et al.*, 1944; Johnson *et al.*, 1945b), pantothenic acid (Cornbleet *et al.*, 1943; Tennet and Silber, 1943; Spector *et al.*, 1945), vitamin B_6 (Johnson *et al.*, 1945e), *p*-aminobenzoic acid (Johnson *et al.*, 1945d), folic acid (Johnson *et al.*, 1945c), inositol (Johnson *et al.*, 1945d), and choline (Johnson *et al.*, 1945a). Figures for the fecal excretion of unabsorbed vitamins are of value only from the explanatory viewpoint. Interpretation of the significance of the quantities of vitamins found in the feces is frequently difficult due to intestinal synthesis by the bacterial organisms, probably in the cecum. This will be apparent from the many papers cited in a later section of this report, demonstrating that intestinal synthesis can be a major factor responsible for the presence of many vitamins in the feces. However, it has proved to be possible to conduct physiological availability studies under controlled conditions so that any increase in the fecal excretion of the vitamin in question can be interpreted as due to unabsorbed dietary vitamin. When this is correlated with the failure of the vitamin to be excreted in the urine, the decreased availability of the nutrient can be definitely attributed to failure of absorption. Parsons and her collaborators (Parsons and Collord, 1942; Parsons *et al.*, 1942; Gardner *et al.*, 1945; Parsons *et al.*, 1945b) have emphasized the value of fecal data to support conclusions drawn from the urinary excretion values. The same general technic has been employed in a large number of studies with human subjects concerned with the physiological availability of the nutrients synthesized by the bacterial flora in the intestinal tract (Oppel, 1942; Najjar and Holt, 1943; Ellinger and Coulson, 1944; Najjar *et al.*, 1944; Alexander and Landwehr, 1945; Benesch, 1945; Ellinger and Benesch, 1945; Gardner *et al.*, 1945; Denko *et al.*, 1946; Hathaway and

TABLE II

Composition of Basal Diet

Breakfast

1 orange	150 g.	2 butter squares	14 g.
2 slices of toast (enriched)	50 g.	1 glass milk	230 g.

Dinner

1 steak (lean)	150 g.	5 butter squares	35 g.
1 serving of fried potatoes	65 g.	1 glass of milk	230 g.
1 serving of carrots	60 g.	1 serving of apple pie	155 g.
1 serving of beets	70 g.	2 hard sugar candies	12 g.
2 slices of toast (enriched)	50 g.		

Supper

2 fried eggs	90 g.	1 glass of milk	230 g.
1 lettuce serving	25 g.	1 apple	150 g.
1 tomato serving	70 g.	1 banana	150 g.
2 slices of bread (enriched)	66 g.	2 hard sugar candies	12 g.
4 butter squares	28 g.		

Analyses Conducted on Aliquots of the Composite Diet

Proximate analysis	Values found[1]	Vitamin content	Values found[1]
Total weight	2075 g.	Thiamine	1.34 mg.
Total solids	540 g.	Thiamine: Calorie ratio	0.5
Moisture	1535 g.	Thiamine: Non-fat calorie ratio	0.9
Protein	101 g.	Ascorbic acid	115 mg.
Fat (ether extract)	129 g.	Riboflavin	2.54 mg.
Ash	22 g.	Niacin[2]	22.0 mg.
Crude fiber	6 g.		
Carbohydrate (by difference)	282 g.		
Caloric value	2710 Cal.		
Non-fat calories	1550 Cal.		

[1] Expressed in terms of total food consumed in the 3 meals.

[2] Predominantly as the amide.

Lobb, 1946; Hathaway and Strom, 1946; Najjar *et al.*, 1946), and in evaluation of vitamin-vitamin and vitamin-amino acid interrelationships (Melnick *et al.*, 1942; Klopp *et al.*, 1943) on the biological response. The urinary excretion procedure has also been used extensively in studies of vitamin availability to experimental animals; these studies are mentioned in later sections of this chapter.

It is not possible within the limitations of this review to evaluate critically all the technics employed for determining the physiological availability of the vitamins using humans as the test subject. Only the procedure developed by Melnick, Hochberg and Oser (1945a) will be discussed in some detail. This method of assay is by far the simplest of all those employed, both from the standpoint of the analyst and that of the test subject, has a degree of precision equal or superior to that of most animal assays, and its reliability has been confirmed by studies conducted in other laboratories (Furter *et al.*, 1946; Kline, 1946; Parsons, 1946).

It has been reported from our own and other laboratories that the urinary excretion of the water-soluble vitamins, as such or as their derivatives, parallels the quantity consumed, provided that normal test subjects are employed and that at the time of the test they are subsisting on an adequate diet. This simple concept is the basis of the technic for measuring the availability to humans of the water-soluble vitamins.

The linear relationship of excretion to dosage is established for a group of experimental subjects, previously shown by analyses of the urinary excretions to be in a good state of nutrition, by feeding graded doses of the vitamins in pure solution. The test dose of vitamin is taken immediately after the largest meal of the day. During this test period of two days, the subject subsists on a normal adequate basal diet of the composition shown in Table II. The first 24-hour urine sample obtained prior to dosage constitutes the basal urine sample. The second 24-hour sample following the postprandial ingestion of the extra vitamin in pure solution is corrected for the basal excretion value of the previous day.[1] Fig. 1 presents the results of a series of such urinary excretion studies of thiamine, riboflavin, niacinamide and ascorbic acid. To determine the physiologically available vitamin content of a test material, the experiment is repeated after an adjustment period of approximately two weeks, feeding an amount of the test substance that (according to chemical or microbiological assay) furnishes a critical dose of the vitamin in question. The ratio of the extra urinary excretion of the vitamin during this period to that following pure solution dosage with the same total quantity of the vitamin, is the index of physiological availability. Since a straight line relationship exists between dosage and urinary excretion in the range

---

[1] Under these conditions of test the extra vitamin excretion is not as great as when the same test dose is taken daily for a period of time. Light and associates (1938), in studies with the dog, reported that excretion equilibrium was attained with increasing thiamine intake provided the dosage was maintained at a given level for a sufficient interval. The same situation was noted in studies conducted with humans subsisting on rations supplemented with large quantities of thiamine, riboflavin, nicotinic acid, and ascorbic acid (Melnick *et al.*, 1942). However, it has been found that the attainment of excretion equilibrium for purposes of measuring physiological availability of the vitamins is unnecessary.

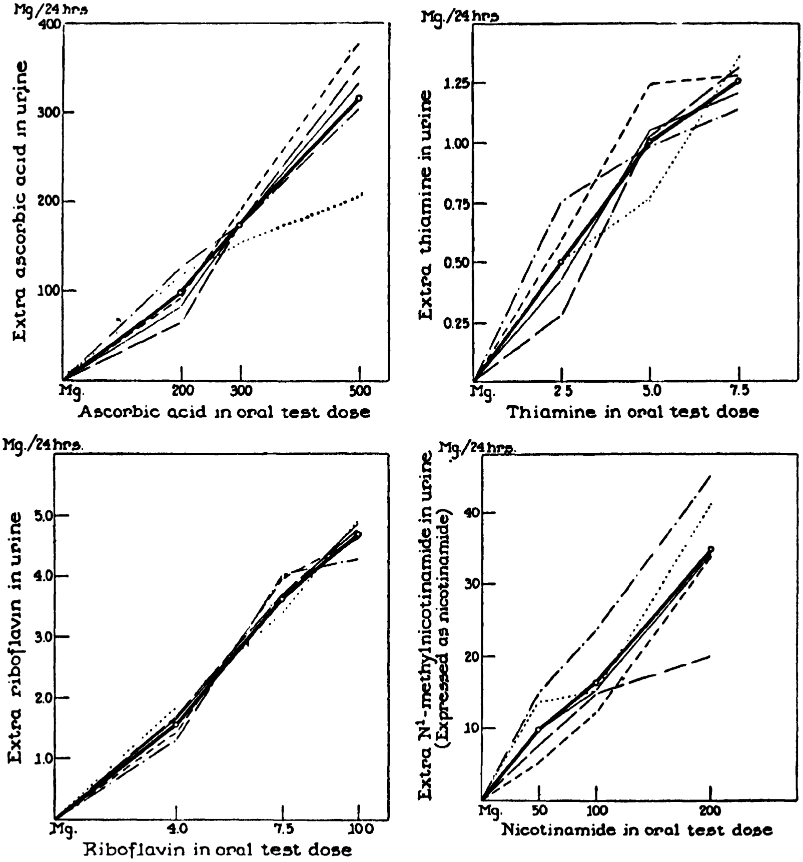


FIG. 1.—The linear relationship between dosage with the water-soluble vitamins, ascorbic acid, thiamine, riboflavin and nicotinamide, and the extra urinary excretion of the vitamins (or derivative). The test doses in aqueous solution (pH 3.0) were taken orally immediately after the mid-day meal. The fine lines represent the responses of the individual subjects whereas the heavy line indicates the average response. (From Melnick, D., Hochberg, M., and Oser, B. L., *J. Nutrition* **30**, 75 (1945).)

of dosage selected, this method of calculating percentage availability is valid.

Any reliable method may be employed for the analysis of the urine samples. It is worth noting that thiamine is excreted in the urine as the free, unphosphorylated vitamin; ascorbic acid is largely, but not entirely, in the reduced state, so that reduction of the dehydro form is necessary; riboflavin is excreted as the free vitamin; while nicotinamide is excreted in large measure as the metabolite N^1-methylnicotinamide.[2] Experimental support has been presented (Melnick *et al.*, 1945a) to justify subtracting the basal excretion values of the first day from the total figures of the subsequent day to arrive at the quantity of extra urinary vitamin (or derivative) in the urine following dosage. It has also been shown that the urinary responses are reproducible to within ±10% of the average (precision superior to that of most biological assays); that in assaying some products (pharmaceuticals) with standardized subjects, the control period is not necessary, thereby reducing the experimental period to only 2 days; that it is possible to conduct simultaneous assays for the 4 water-soluble vitamins thiamine, ascorbic acid, riboflavin and nicotinamide; that by collecting urine samples at periodic intervals to evaluate rate as well as degree of vitamin availability; and that all of these are obtainable with human subjects rather than with animals in a pathological state.

The motivating factor in the development of the human bioassay technic was the unreliability of *in vitro* procedures (Brenner, 1943; Federal Surplus Commodities Corp., 1944; Thompson and Lee, 1945a; 1945b) for determining the physiological availability of the vitamins in pharmaceutical tablets and capsules. Obviously, rat assays could not be employed for this purpose while larger laboratory animals which are capable of swallowing intact tablets or capsules likewise can not be used since the vitamin intake would far exceed the critical range of dosage in the conventional prophylactic or curative bioassays. The successful use of the human bioassay technic in testing the availability of the vitamins in pharmaceutical products has been reported by other laboratories (Rubin, 1946; Kline, 1946).

Determinations of the physiological availability of the vitamins employing this procedure have since been reported for pharmaceutical tablets in which the vitamin ingredients are protectively coated to insure their stability (Oser *et al.*, 1945); for the thiamine in live yeast cells

---

[2] The recent finding that pyridone (N^1-methyl-6-pyridone-3-carboxylamide) is also a major excretory product (Knox and Grossman, 1946) does not vitiate this assay method since a linear dose-response relationship exists between nicotinamide intake and excretion of N^1-methylnicotinamide.

(Hochberg *et al.*, 1945c); for ascorbic acid as influenced by the presence of copper (Hochberg *et al.*, 1945d) and of ascorbic acid oxidase (Hochberg *et al.*, 1945b); for thiamine as influenced by the presence of the antithiamine factor in raw sea-foods (Melnick *et al.*, 1945b); and for thiamine and riboflavin in the presence of adsorbing agents (Melnick *et al.*, 1945c; Oser *et al.*, 1945). The unreliability of *in vitro* technics in general in predicting the availability of vitamins has also been demonstrated in these studies.

It has been assumed by some workers that the human assay technic can be used in evaluating the availability of vitamins only when they are present in very large quantity, *i.e.*, in multiples of the minimal daily requirements. However, this is not necessarily so. By slight modification in the basal dietary and extending the basal collection period, it has proved possible to determine the effect of various factors upon the availability of *dietary* thiamine (Melnick *et al.*, 1945b) and of *dietary* ascorbic acid (Hochberg *et al.*, 1945b). Experiments along such lines have been reported by others (Ness *et al.*, 1946).

TABLE III

Urinary Excretion of Dietary and Extra Thiamine by Subjects Subsisting on a Diet of Raw and Heated[1] *Clams*

(All values in mg.)

Subject	Diet containing heated clams					Normal basal excretions[2]	Diet containing raw clams[3]				
	Basal excretions			After 7.5 mg. of thiamine			Basal excretions			After 7.5 mg. of thiamine	
	1st 24 hrs.	2nd 24 hrs.	3rd 24 hrs.	1st 24 hrs.	2nd 24 hrs.		1st 24 hrs.	2nd 24 hrs.	3rd 24 hrs.	1st 24 hrs.	2nd 24 hrs.
J. C.	0.22	0.23	0.23	1.59	0.54	0.23	0.12	0.11	0.10	0.88	0.16
E. M.	0.25	0.19	0.19	1.33	0.33	0.21	0.12	0.09	0.07	0.59	0.25
D. M.	0.25	0.29	0.28	1.80	0.47	0.27	0.17	0.11	0.11	0.74	0.25
M. H.	0.19	0.24	0.22	1.02	0.38	0.22	0.15	0.14	0.11	0.85	0.25
H. H.	0.32	0.28	0.26	1.79	0.36	0.29	0.14	0.09	0.09	0.95	0.18
Average	0.25	0.25	0.24	1.51	0.42	0.24	0.14	0.11	0.10	0.80	0.22

[1] An aqueous suspension of ground clams at pH 4.5 boiled to inactivate the thiaminase.

[2] The average excretion of the corresponding subject subsisting on the diet containing the heated clams.

[3] The raw clams were served whole.

For illustrative purposes the results of one availability study are presented in some detail. This was concerned with the effect of a dietary thiaminase in fish products on the physiological availability of the thiamine. The experimental findings are presented in Table III. The control data were obtained with the subjects on the adequate basal ration plus clams heated to inactivate all the enzymes present. During the first 3 days no extra thiamine was taken; on the fourth day a test dose of 7.5 mg. of the vitamin was administered, and on the fifth day the basal ration alone was again ingested. The uniformity of response of the various subjects is apparent. The subjects show excellent reproducibility from day to day in basal thiamine excretions, elevated values following the taking of the test dose with small carry-over values. Two weeks later the experiment was repeated. This time the same quantity (100 g.) of clams was eaten in the raw state. On the first day there was a marked fall in the basal urinary excretion values and these figures continued to decrease progressively. When the test dose of 7.5 mg. of thiamine was

TABLE IV

Destruction of Dietary and Extra Thiamine by Thiaminase in Clams

Subject	Diet containing heated clams			Diet containing raw clams				
	Average basal excretion, mg./24 hrs.	Fraction of 7.5 mg. test dose excreted		Change in basal excretion[1]			Fraction of 7.5 mg. test dose excreted	
		1st 24 hrs., %	2nd 24 hrs., %	1st 24 hrs., %	2nd 24 hrs., %	3rd 24 hrs., %	1st 24 hrs., %	2nd 24 hrs., %
J. C.	0.23	18.1	4.1	−46	−50	−56	10.3	0.8
E. M.	0.21	14.8	1.5	−44	−56	−66	6.9	2.3
D. M.	0.27	20.4	2.6	−36	−58	−60	8.4	1.9
M. H.	0.22	10.8	2.2	−30	−37	−48	9.8	1.8
H. H.	0.29	20.0	0.9	−53	−68	−68	11.4	1.2
Average	0.24	16.8	2.3	−42	−55	−60	9.4	1.6
Average total excretion of extra thiamine		19.1%					11.0%	
Destruction of thiamine taken as test dose	$\frac{19.1 - 11.0}{19.1} \times 100 = 42\%$							

---

[1] Based on the average excretions of the corresponding subjects receiving the diet containing the heated clams.

superimposed on the basal diet much smaller responses were obtained as compared with the control values. This was also true in the case of the carry-over figures.

Table IV is an evaluation of the data in the preceding table on the effectiveness of the thiaminase in destroying both dietary and extra thiamine. Approximately 50% of the dietary thiamine was destroyed. When the urinary responses, expressed as per cent of the test doses excreted during the control and test periods, are compared we find that here also about half of the extra thiamine taken was destroyed.

It will be shown in succeeding sections of this report that various factors are capable of enhancing or diminishing the biological response to ingested vitamins. Vitamin-vitamin and vitamin-amino acid interrelationships, intestinal synthesis or destruction of the vitamins, concomitant ingestion of antivitamins, are factors which must be considered in the rating of dietary sources of any particular vitamin.

IV. FACTORS INFLUENCING THE PHYSIOLOGICAL AVAILABILITY OF THE VITAMINS

There is little reason, from the standpoint of structural similarity or functional interrelationship, to continue to classify vitamins on the basis of their solubility in water or fat. However, there are conditions in which solubility characteristics influence the physiological availability of vitamins and therefore they will be considered as they apply to groups of vitamins generally.

1. Fat-Soluble Vitamins

The amount and nature of the fat in the diet, or the oily menstruum in which fat-soluble vitamins are administered, influence the extent to which they are available for absorption from the intestinal tract. The administration of critical doses of these vitamins dissolved in paraffin oil has long been known to interfere with their absorption because of the preferential solubility of the vitamin in such nondigestible vehicles. This has been shown for vitamin A (Rowntree, 1931), carotene (Curtis and Kline, 1939), vitamins D, E and K (J.A.M.A., 1946). The magnitude of this interference has often been over-emphasized as a nutritional problem, since much of the experimental basis is derived from studies in which excessive quantities of paraffin oil were administered concomitantly with minimal doses of vitamins. In the case of certain special dietary foods containing inert hydrocarbon oils it has been found possible (Oser and Oser, 1946) to add vitamins A and D to an extent sufficient to provide nutritionally adequate quantities of these vitamins.

The absorption of vitamin A by previously depleted rats was aided by dietary fat, although its subsequent utilization was independent of the fat (Muelder and Kelly, 1942). In the chick, the level of dietary fat was without effect on the absorption of preformed vitamin A, but the presence of fat in the ration markedly improved the absorption of carotene (Russell *et al.*, 1942). Lecithin freed from neutral fat has been reported to improve the absorption of vitamin A even in patients with sprue (Adlersberg and Sobotka, 1943). The effect of dietary fat on absorption of other fat-soluble vitamins has not received as much study. However, the opposite effect of an increased requirement for a vitamin when the diet is rich in fat has been shown in the case of vitamin E (Gottlieb *et al.*, 1943).

The ability to absorb esterified vitamin A from its natural oily vehicle is impaired in children with celiac disease (Sobel *et al.*, 1946) but that absorption can occur when the vitamin, as the free alcohol, is administered orally in aqueous dispersion is shown by a rise in blood vitamin A levels comparable to that observed in normal children.

Pathological conditions affecting intestinal absorption of vitamins come into the category of conditioning factors described by Jolliffe (1943) which are contributory causes of vitamin deficiencies. However, these factors can operate to affect the physiological availability of vitamins under pathological conditions, and for this reason are mentioned in the present paper.

The role of bile or bile salts in the absorption of fats is manifested in the incomplete absorption of fat-soluble vitamins in hepatic dysfunction, obstructive jaundice, or other conditions in which biliary flow is impaired. Thus, the presence of bile salts is essential for the absorption of carotene (Greaves and Schmidt, 1935), vitamin D (Greaves and Schmidt, 1933; 1934), vitamin E (Greaves and Schmidt, 1937) and vitamin K (Greaves, 1939); and, while not required for vitamin A absorption (Schmidt and Schmidt, 1930), bile salts may accelerate it (Clausen, 1938).

Beside those conditions, mentioned above, which affect the availability of the fat-soluble vitamins generally, individual vitamins in this group are subject to specific influences which may alter their stability or absorption *in vivo*.

a. Vitamin A. It has been shown that preformed vitamin A has about the same biological activity whether administered in alcohol or ester form (Kringstad and Lie, 1944). In addition to the presence of bile salts, as discussed above, conditions necessary for the absorption of vitamin A esters, the principle form in liver oils, are normal intestinal mobility (May and McCreary, 1940) and the presence of an unknown factor in pancreatic secretion (Clausen *et al.*, 1946). Children with cystic fibrosis of the pancreas, who are deficient in pancreatic enzymes, can absorb vitamin A alcohol but not the ester unless it is supplemented with pancreatin.

In dietaries containing insignificant quantities of antioxidants, the addition of tocopherols gives an enhanced vitamin A effect (Hickman and Harris, 1946). This was noted in bioassays using either preformed vitamin A or carotene as the test material. The effect of the tocopherol is attributed to protection of the vitamin against oxidative destruction both in the gastrointestinal tract prior to absorption and at the site of utilization within the organism proper, but principally the former.

Greater amounts of carotene and other oxidizable substances can be recovered in the feces following administration of tocopherol simultaneously with the carotene. α-, β- and γ-Tocopherol appear to be equally effective in their antioxidant activities, but there is some evidence that a mixture of tocopherols may be more active than any one alone. The free tocopherols are more effective than their esters (Hickman and Harris, 1946).

Because improperly controlled bioassays can lead to erroneous estimations of physiological availability, it may be pointed out that when a basal vitamin A-free diet is uncontrolled as to tocopherol content, the response to an unknown may be the resultant of two factors, vitamin A and tocopherol content. Under such conditions the assay is no longer specific for vitamin A. It is of interest to note that ascorbic acid may play a synergistic role with the tocopherols (Golumbic and Mattill, 1941). Even without tocopherol, aqueous colloidal solutions of carotene exhibit greater stability on the addition of ascorbic acid (Karrer and Straus, 1938).

Utilization of carotene by the rat has been shown to be a function of the solvent in which it is administered (Lease *et al.*, 1939; Sherman, 1941). By means of comparative tests based on liver storage and fecal excretion in rats, it has been found that carotene, when fed in the form of vegetables, has relatively less biological value than when fed in butterfat or beef liver (Fraps and Meinke, 1945). It has also been observed in man that absorption of carotene from carrots is less efficient than from butterfat (Kreula and Virtanen, 1939).

Similar observations with respect to the comparative biological responses to carotene in oil (*e.g.*, the International Vitamin A Standard) and analytical values for carotene in vegetables emphasize the risk involved in assuming that estimates of vitamin A based on the relation 0.6 γ β-carotene = 1 unit of vitamin A, are measures of true biological activity. In this connection the relative purity of the analytically determined "carotene" and that contained in the International Standard must be considered as well as the degree of absorption of carotene from the test material. In the case of certain canned (heat-processed) vegetables, better agreement with values obtained by bioassay (in which the tocopherol content of the diet was controlled) was found with the assumed relation of 1.0 γ crude carotene = 1.0 unit of vitamin A activity (Oser and Melnick, 1945). In studies of the vitamin A content of certain dehydrated vegetables (Wilson *et al.*, 1946) it was found that the rat has difficulty in extracting all the carotene, so that the figures obtained by the biological tests were considerably less than those yielded by the physical method of assay.

Without correcting for the tocopherol variable in the dietary, Guggenheim (1944) demonstrated that the utilization of carotene derived from various plant materials ranged between 33 and 100% of that noted when preformed vitamin A was taken. In other studies with the rat, substitution of casein for corn meal in the ration increased the percentage absorption of carotene from the digestive tract (Fraps, 1946). Inasmuch as the efficiency of conversion of carotene to vitamin A is variable from species to species (Ahmad and Malik, 1933), it must be cautioned that the results obtained with test animals are not necessarily directly applicable to man.

b. Vitamin D. Since the presumption is prevalent that standard bioassays for vitamins may be regarded as indices of physiological availability, it is relevant to point out some of the factors that influence bioassay results. In the case of vitamin D the amount of phosphate is a critical factor in determining the response of the rachitic rats to variable vitamin D intake (Shohl, 1936). Thus, in assaying sources of vitamin D which likewise contains phosphorus (*e.g.*, milk, animal feeds, vitamin-mineral concentrates) adequate provision must be made to control the Ca:P ratio and content of the diet (A.O.A.C., 1945). The response to vitamin D is also influenced by the content of phytin in the diet. The effect of the Ca:P ratio on vitamin D requirement varies with different species, and is not the same in the infant as in the rat. Starvation causes a remobilization of body calcium in the rat and produces apparent healing of the rachitic lesion (McCollum *et al.*, 1921), thus necessitating the insurance of adequate food intake in vitamin D assays. Furthermore, certain fatty acids have a slight antirachitic value, thus reducing the requirement for vitamin D (Booth *et al.*, 1942).

c. Vitamin E. The inclusion of sulfaguanidine and sulfasuxidine in diets marginal in vitamin E content produces hyalinization, necrosis and calcification of voluntary muscle in rats (Daft *et al.*, 1942). An oral supplement of 3 mg. of α-tocopherol per week prevented the appearance of the lesion (Daft *et al.*, 1943). Whether or not this indicates synthesis of vitamin E by intestinal bacteria is still uncertain. Vitamin E is not normally present in feces (Hines and Mattill, 1943) and does not appear to be synthesized by microorganisms in the rumen of cows (Goss, 1943).

d. Vitamin K. The principle clinical causes of reduced physiological availability of vitamin K are biliary hyposecretion or obstruction. In these conditions, concomitant administration of bile salts, or the use of water-soluble derivatives of menadione effectively improve the characteristic hypoprothrombinemia.

Among the fat-soluble vitamins, vitamin K is the major factor synthesized to a significant degree by intestinal flora. Hence, conditions which diminish or prevent bacterial growth in the intestinal tract may reduce the amount of available vitamin K below safe levels.

It has been observed, for example, that the clotting time of the blood was increased in the presence of succinylsulfathiazole and that this change could be counteracted by the addition of vitamin K (Black *et al.*, 1942).

Ordinarily the requirement of the rat for vitamin K can be met through intestinal synthesis; a deficiency can be induced, however, by the feeding of sulfasuxidine. This condition responds favorably to the supplementation of the diet with folic acid and biotin even in the absence of a dietary source of vitamin K (Welch and Wright, 1943). Presumably, synthesis of the latter vitamin results from stimulation of the growth of bacteria which are not too sensitive to the inhibitory action of the sulfonamide (Poth *et al.*, 1941).

Chicks are particularly sensitive to dietary lack of vitamin K but nevertheless, the characteristic hemorrhagic syndrome fails to develop in some chicks, while in others spontaneous recovery occurs even when the chicks are allowed to continue on the deficient ration (Almquist and Stokstad, 1936). This has been ascribed to the fact that intestinal synthesis in these animals fluctuates closely around their minimal requirement, so that birds within a given group may show different responses.

2. Water-Soluble Vitamins

The property of solubility in water which characterizes this large group of vitamins has an important bearing upon their physiological availability since ingested vitamins may be exposed to enzymes, pH changes, adsorbents, *etc.*, which operate most effectively in aqueous medium. The water-soluble vitamins differ from the fat-soluble vitamins in that they are excreted *via* the kidneys and to a small extent *via* the sweat glands. Thus, circulatory or secretory factors may be expected to play a role in determining their degree of retention or rate of elimination. The fact of their excretion by the urinary route provides a means of studying the physiological availability of this group of vitamins.

It is also worthy of note that so far as we know today, the vitamins which are synthesized by intestinal flora to any extent are in the water-soluble group; though vitamins E and K are likewise produced by bacterial synthesis, it has not been determined whether they are produced in fat- or water-soluble form.

a. Ascorbic Acid. The susceptibility of this vitamin to atmospheric and enzymic oxidation is a factor to be considered in relation to its availability.

Despite the fact that *in vitro* ascorbic acid oxidase rapidly destroys large quantities of ascorbic acid, no great destruction of either the naturally occurring, dietary ascorbic acid or of extra ascorbic acid was noted *in vivo* due to this factor (Hochberg *et al.*, 1945b). Apparently ascorbic acid oxidase in the fresh fruit and vegetables consumed is destroyed or its activity is inhibited in the gastrointestinal tract. It has been demonstrated that ascorbic acid oxidase can rapidly oxidize ascorbic acid in vegetables to and beyond the dehydroascorbic stage. The catalytic effect of copper upon the oxidative destruction of ascorbic acid is well recognized. However, human availability studies have indicated (Hochberg *et al.*, 1945d) that, in the digestive tract, ascorbic acid and copper are not incompatible. No greater destruction of the ascorbic acid occurred *in vivo* as a result of the administration of extradietary copper.

Studies have suggested that the capillary permeability factor, vitamin P, identified as a mixture of flavone glycosides (hesperidin, eriodictin, *etc.*) is required for the cure of certain of the hemorrhagic manifestations associated with scurvy. The evidence, which has been recently reviewed (*Nutrition Revs.*, 1943 and 1944), rests largely upon the demonstration that natural foods high in ascorbic acid and containing these flavones, are more effective therapeutically than equivalent amounts of synthetic ascorbic acid. On the other hand, rather than attribute the greater efficacy of natural foods to specific functions of vitamin P in supplementing those of ascorbic acid, it has been postulated (Elmby and Warburg, 1937) that foods furnish stabilizing factors which promote a more effective utilization of ascorbic acid. This hypothesis has been supported by Crampton and Burton (1946) who believed their newly developed bioassay procedure to be specific for ascorbic acid. They reported that the juices of the orange, apple, and tomato exhibit about 35–45% more vitamin C potency than is indicated by the chemical assay for total ascorbic acid. The presence in plant products of factors which inhibit oxidative destruction of ascorbic acid has been noted (Somogyi, 1945; Reder, 1946). Factors capable of reversing the partial oxidation of ascorbic acid have also been found in natural foods. Hopkins and Morgan (1936) reported the presence in cauliflower juice of an enzyme that catalyzes the reduction of dehydroascorbic acid by glutathione, thus enhancing the protective action of glutathione toward the active vitamin. Though this could not be confirmed by Kertesz (1938), enzymic catalysis of dehydroascorbic acid reduction in certain plant juices was corroborated by Crook and Hopkins (1938) and subsequently the former (Crook, 1941) was able to separate the enzyme from ascorbic acid oxidase. The role of glutathione in the reduction of dehydroascorbic acid in animal tissues was demonstrated by Borsook and others (Borsook *et al.*, 1937) and by Schultze and associates (1938) who also showed that acid-insoluble SH compounds behave similarly.

In previous studies of the availability of ascorbic acid in orange juice (Hawley *et al.*, 1936), in raspberries (Todhunter and Fatzer, 1940), in potatoes (Clayton and Folsom, 1940), in cabbage and tomato juice (Clayton and Borden, 1943) and in papayas and guavas (Hartzler, 1945), no differences in the urinary excretion values were noted when the vitamin was furnished in its natural medium of in pure solution. In these studies, however, the basal dietaries were not devoid of other fruits and vegetables; despite the necessity to reduce irrelevant ascorbic acid to a minimum, the unsupplemented rations supplied from 5 to 20 mg. of the vitamin daily. Since vegetable extracts, even those low in ascorbic acid, contain stabilizing substances which could conceivably enhance biological potency (Somogyi, 1945; Reder, 1946), the synthetic ascorbic acid taken with the basal dietaries might also have been subjected to the protective action of the same type of stabilizers as found in the test fruits or vegetables. In this connection it is significant that the protective factor is not destroyed by boiling the vegetable extracts (Reder, 1946). This phase of ascorbic acid availability merits further study.

It has also been reported that the utilization of ascorbic acid in some children was enhanced by the injection of potassium citrate (Meyer and Hathaway, 1944). The effect was even greater when orange juice was used in place of the crystalline vitamin.

That conditions in the intestinal tract may influence the absorption or destruction of vitamin C is indicated by various studies. Cases of clinical scurvy sometimes are refractory to oral administration of the vitamin but respond to intramuscular injection (Young and James, 1942; Young and Rettger, 1943). Cultures of normal intestinal bacteria isolated from human stools have been shown to destroy ascorbic acid under both aerobic and anaerobic conditions. Glucose or lactose in the media exerted a sparing action. Human feces normally contain but little ascorbic acid even when large amounts are ingested (Young and James, 1942; Young and Rettger, 1943). Hypoacidity due to achlorhydria or to the use of alkalizing agents may interfere with the absorption or stability of ascorbic acid (Einhauser, 1936; Field *et al.*, 1940).

Certain evidence suggests that other vitamins or amino acids may affect the metabolism of ascorbic acid. When a mixture of niacin (or niacinamide) and ascorbic acid is moistened and ground to a paste, a reaction occurs with the formation of a yellow end-product (Milhort, 1944). No change is observed in the reducing properties of the ascorbic acid, but whether or not the biological activity is unaltered remains to be shown.

When guinea pigs or infants in a state of ascorbic acid deficiency ingested extra amounts of phenylalanine or tyrosine, partial metabolites of these amino acids were excreted in the urine. Administration of small doses of ascorbic acid corrected this metabolic anomaly (Basinski and Sealock, 1946). The onset of scurvy in guinea pigs on vitamin C-free rations, has been found to be hastened by the administration of extra tyrosine (Sealock, 1945).

b. Thiamine. It has long been recognized that the caloric, and more specifically the non-fat caloric, intake is an essential factor in determining the thiamine requirement. Increasing the carbohydrate intake results in diminution of the urinary output of thiamine (Reinhold *et al.*, 1944), indicating a greater need for this vitamin in metabolism. Marked increases in fat consumption fail to increase the urinary excretion values (Cahill, 1941; Reinhold *et al.*, 1944) despite the fact that in experimental animals a high fat diet reduces the requirement for thiamine (Evans and Lepkovsky, 1929; Arnold and Elvehjem, 1939; Stirn *et al.*, 1939). The thiamine content of the tissues varies with vitamin intake and unlike some of the other B-factors, *viz.*, riboflavin and nicotinic acid, is independent of protein intake. It has been reported (Williamson and Parsons, 1945) that human subjects ingesting a high proportion of cellulosic fiber (pineapple) excrete larger amounts of thiamine *via* the stools although the urinary excretion remains unchanged. This suggests that the fiber does not interfere with the absorption of dietary thiamine but actually stimulates intestinal synthesis of the vitamin. However, the latter fails to be absorbed from the digestive tract.

In hyperthyroidism the need for thiamine is definitely increased (Himwich *et al.*, 1931–32). This was shown, for example, by the fact that on a B-deficient diet anorexia appeared in one-half to two-thirds the time required during the control period. Human subjects with hyperthyroidism subsisting on an adequate ration excrete very little thiamine in the urine when the caloric intake is maintained at 2500–3000 calories (Robinson *et al.*, 1940).

The form in which thiamine is consumed, as well as concomitant medication, play an important role in determining the efficiency with which it is absorbed in the digestive tract. Before crystalline thiamine became available, a fullers' earth adsorbate of an extract of rice polish was used as the International Standard for vitamin B_1. This preparation was subsequently shown (Keresztesy and Sampson, 1937) to be unsatisfactory as a reference standard since it actually contained twice as much thiamine as was believed to be present, the animal organism being able to elute only one-half. Fullers' earth, kaolin, or similar adsorbents taken regularly or in appreciable quantities over long periods for the treatment of various clinical disorders were found (Melnick *et al.*, 1945c) to react in an unpredictable manner in the organism. Whereas the availability of thiamine was markedly reduced by the concomitant ingestion of fullers' earth, there was no interference when kaolin was taken along with thiamine. *In vitro* tests proved unsatisfactory for anticipating the influence of an adsorbent on thiamine availability (Melnick *et al.*, 1945c). Peptic ulcer patients receiving antacid medication usually excrete only small amounts of thiamine in the urine (Robinson *et al.*, 1940). Increased alkalinity in the gastrointestinal tract favors greater destruction of thiamine, while adsorbents, particularly magnesium trisilicate, cause a loss of thiamine to the organism by rendering the vitamin unavailable for absorption (Melnick *et al.*, 1941).

An acute dietary deficiency has been produced in animals by including uncooked fish in the dietary (Green *et al.*, 1942); this could be prevented or cured by giving adequate amounts of thiamine. The factor responsible for this effect was demonstrated to be an enzyme (Krampitz and Woolley, 1944; Sealock and Goodland, 1944) found generally only in fresh water fish (Deutsch and Hasler, 1943). The presence of an active thiaminase in clams has also been reported (Melnick *et al.*, 1945b). In availability studies with human subjects, cited as an illustration in Tables III and IV (Section III, 2 above), it was found that appreciable, destruction of thiamine (about 50%) occurred in the gastrointestinal tract following the concomitant ingestion of raw clams. The destructive enzyme was also found in the Atlantic herring, but was absent in oysters.

Certain cereals, legumes and oil seeds have been found to contain a non-enzymic factor capable of destroying thiamine (Bhagvat and Devi, 1944); it is extracted by chloroform-water mixture and stable to heat, including autoclaving. As in the case of sulfite cleavage, the end-products of this destruction are believed to be pyrimidine and thiazole derivatives.

The possibility that other plant materials may be toxic by virtue of their deleterious effect on thiamine is suggested by studies with a bracken fern growing in an area where fern-poisoning of cattle occurred. When this plant was fed to rats, symptoms suggestive of thiamine deficiency were observed (Weswig *et al.*, 1946). The solubility of the factor causing thiamine destruction in 92% ethanol indicates that it is non-enzymic; furthermore, no decrease in toxicity resulted when the dried fern was heated in air at 105°C. for 18 hours.

The cellular structure, or rather the composition of the cell wall, may have an important bearing on the extent to which thiamine is released for absorption. For example the vitamins of the B-group, and especially thiamine, are not readily absorbed from the gastrointestinal tract when fresh yeast is the sole source of this vitamin (Walker and Nelson, 1933; Parsons *et al.*, 1945a); this has been attributed to the resistance of live yeast cells to digestion. Availability studies with human subjects (Hochberg *et al.*, 1945c; Parsons *et al.*, 1945) demonstrated that only a small fraction of the thiamine in live yeast was available for absorption. Thiamine not accounted for by the urinary excretion during the period of fresh yeast ingestion was largely present in the feces. Rupturing the yeast cell by suspending the material in boiling water made the thiamine available to the human (Parsons *et al.*, 1945b). In a subsequent study (Ness *et al.*, 1946) it was found that the body stores of thiamine were decreased during the live yeast feeding period as evidenced by the urinary excretion values. Not only was the thiamine contained within living yeast cells not absorbed, but the yeast was able to render unavailable the thiamine supplied by other items in the dietary. The capacity of viable yeast to withdraw thiamine from a surrounding medium has also been demonstrated by *in vitro* experiments (Hochberg *et al.*, 1945c). These findings demonstrate that reduced thiamine availability is not due to the more rapid transit of food through the digestive tract attributable to increased gastrointestinal motility following the ingestion of fresh yeast (Russell and Nasset, 1941).

The early literature on the phenomenon of refection in rats contains repeated references to the synthesis of vitamin B by intestinal flora and the effect of dietary carbohydrates on this process. Interest in the nutritional significance of bacterial synthesis was revived with the demonstration that the rat, among other species, could obtain a sufficient supply of some of the B-vitamins from this source to make it independent of dietary intake of these factors. Little is known of the extent to which the human organism, under various physiological, pathological or nutritional conditions, can satisfy its vitamin needs through the intervention of bacterial processes in the digestive tract.

When the rat ingests dextrinized cornstarch as the sole carbohydrate, bacterial synthesis of thiamine and of riboflavin is capable of lowering the dietary requirement for these two vitamins (Guerrant and Dutcher, 1934a; 1934b). This is in sharp contrast to the poor synthesis induced by feeding commercial cornstarch, sucrose, glucose or lactose. Comparable observations have been reported by Najjar and his associates on human subjects (Najjar and Holt, 1943; Najjar and Barrett, 1945). For example, dextrimaltose feeding was followed by intestinal synthesis of thiamine, but replacement of this carbohydrate with rice or with sucrose resulted in markedly lower thiamine production. Preliminary dextrinization of starch appeared to favor bacterial synthesis of thiamine. The development of deficiency symptoms was accompanied by a decrease in the fecal output of free thiamine.

These studies were extended by Hathaway and Strom (1946) who found that a purified diet such as that used by Najjar and Holt (1943) was even less favorable than a natural diet for intestinal thiamine synthesis. However, since lowered thiamine intake was reflected in the urinary excretion while, at the same time, fecal excretion of free thiamine was small, it is likely that intestinal synthesis of the vitamin is not a major factor in the thiamine economy of the human. In this connection mention should be made of the studies by Alexander and Landwehr (1945) demonstrating inappreciable absorption of thiamine from the colon. The fat content of the ration can also influence bacterial synthesis of thiamine in the intestinal tract (Whipple and Church, 1935). When lard is added to a thiamine-deficient diet containing sucrose as the carbohydrate, the feces passed by the rat show thiamine activity; when the lard is withheld, thiamine activity of the stools is lost. Other factors also appear to stimulate growth of rats on limited thiamine intake (McIntire *et al.*, 1943).

Various lines of evidence point to the existence of interrelationships among vitamins of the B group. The tissues of thiamine-deficient rats contain diminished concentrations of riboflavin and in chronic thiamine deficiency the urinary excretion of riboflavin is much increased, suggesting poor utilization of the latter vitamin (Sure and Ford, 1942; Sure, 1944). In riboflavin deficiency, on the other hand, a decrease of tissue thiamine is noted but this is not accompanied by a change in urinary excretion. Under these experimental conditions, it was subsequently shown (Singher *et al.*, 1944) that mobilization of riboflavin in the liver takes place and a portion of the transported riboflavin is excreted in the urine. Similarly, mobilization of thiamine occurs when extra riboflavin is administered. Deficiencies of other B-vitamins, including pyridoxine, pantothenic acid and biotin, do not influence the concentration of thiamine or riboflavin in the liver. Under conditions of moderate dosage of vitamins, there appears to be no interaction of thiamine and riboflavin as reflected by their influence on the growth of rats (Cross *et al.*, 1946). This is an important consideration since it bears on the reliability of the technic for estimating the physiological availability of vitamins to humans; in this procedure normal subjects are given moderate doses of the vitamin of interest.

c. Riboflavin. In addition to the interrelationship just described of riboflavin and thiamine, it has been shown in dogs that the urinary excretion of riboflavin is inversely related to the protein intake (Sarett *et al.*, 1942). Rats maintained on a low protein diet excrete so much riboflavin that they fail to maintain a functional level in their tissues and ultimately die of ariboflavinosis despite the administration of relatively large dosage of the vitamin (Czaczkes and Guggenheim, 1946). Increasing the protein or fat content of these diets reduced the riboflavin excretion, while decreasing the fat content raised the excretion. These observations are attributed to variations induced in the tissue levels of riboflavin as influenced by the extent of synthesis of the vitamin by intestinal flora.

It has been shown that the riboflavin requirement of the rat is affected by the fat content of the diet (Elvehjem, 1946). The severity of ariboflavinosis in rats on a dextrin-containing deficient diet could be increased by the isocaloric substitution of fat for a large proportion of the dextrin. That intestinal synthesis is an underlying cause of this observation was shown by the fact that in some instances fecal excretion alone was greater than dietary intake of riboflavin. In other studies, on human subjects, it has been reported that the combined urinary and fecal output was greater than the intake (Davis *et al.*, 1946). As in the case of thiamine synthesis it has been shown (Hathaway and Lobb, 1946) that, in human subjects, natural diets dispose more favorably toward bacterial synthesis of riboflavin in the intestinal tract than the type of synthetic diets employed by the Johns Hopkins investigators (Najjar *et al.*, 1944). The latter also found the daily urinary output on a riboflavin-low diet to be several times the intake.

The nature of the dietary carbohydrate plays a significant role in determining the extent of bacterial synthesis of riboflavin. Dextrin, cornstarch and lactose favor this process whereas sucrose or increased fat content of the diet have an opposite effect (Mannering *et al.*, 1944). Bacterial synthesis of riboflavin can occur in the colon of the rat. The dietary requirement of this species for riboflavin is reduced under conditions favoring bacterial synthesis (Guerrant and Dutcher, 1934a; 1934b; Morgan *et al.*, 1938; Mitchell and Isbell, 1942), although there is some question where in the gastrointestinal tract this synthesis takes place (Griffith, 1935).

The preceding studies, bearing on factors influencing the urinary excretion and requirement of riboflavin, emphasize the need of keeping the subjects in a human availability study on an adequate standardized ration during the period of test.

Absorption of riboflavin from the intestinal tract may be influenced by the cellular form or chemical combination in which it may be ingested. Parsons and associates (Parsons and Collord, 1942; Parsons *et al.*, 1942) have shown, for example, that the riboflavin, like the thiamine, in live yeast is only poorly available. Pharmaceutical multivitamin preparations have been found in which the riboflavin is rendered so insoluble as to be unavailable (Oser *et al.*, 1943a). Derivatives of riboflavin have been prepared with the object of increasing the solubility of the vitamin and hence its availability. For example, several succinate derivatives have been synthesized (Furter *et al.*, 1945) which, though fluorometrically equivalent, had less biological (rat) and still less microbiological activity. The biological and microbiological activity of the succinates decreased with the extent of substitution. It is claimed that neither the fluorometric nor the microbiological activity of such flavin derivatives is an accurate measure of their activity for mammals. These observations emphasize the importance of availability studies in expressing vitamin content.

d. Niacin. Various groups of investigators have presented convincing evidence that the rat is capable of synthesizing niacin (Shourie and Swaminathan, 1940; Dann, 1941; Mitchell and Isbell, 1942; Sarett and Perlzweig, 1943). The site of synthesis may be within the tissues themselves as well as in the colon. The composition of the diet bears an important relation to the extent of niacin synthesis and to the requirement for this vitamin. Rats are able to grow well on a diet containing 9% casein plus all the B vitamins with the exception of niacin. However, replacement of a large part of this diet with corn grits results in retarded growth which can be restored by the addition of niacin to the ration (Krehl *et al.*, 1945a). This might be explained simply by the effect of the diet on the metabolic process involved in bacterial synthesis, were it not for the fact that tryptophan as well as niacin can restore growth under these conditions (Krehl *et al.*, 1945; Krehl, Sarma *et al.*, 1946). From recent paired feeding studies it was concluded that these effects of niacin and tryptophan are due neither to tissue nor bacterial synthesis but possibly to some unknown metabolic process in which both can participate interchangeably (Spector and Mitchell, 1946). Thus, tryptophan either promotes niacin synthesis or reduces the requirement for this vitamin.

In balance studies, it was found (Krehl, de la Huerga, and Elvehjem, 1946) that niacin improves the utilization of tryptophan from 30 to 70%. The substitution of a tryptophan-deficient diet for one containing adequate protein, resulted in lowered excretion of N^1-methylnicotinamide, one of the principal metabolites of niacin; conversely, the addition of tryptophan to an adequate ration prompted a marked increase in excretion not only of the metabolite but even of non-methylated niacin (Rosen *et al.*, 1946; Singal *et al.*, 1946). Fecal excretion of niacin or its metabolite was not significantly affected by tryptophan administration, indicating that the amino acid may be the important precursor or sparer of niacin in the rat and that bacterial synthesis in the gut is of only secondary importance.

In the pig on niacin-deficient rations, a relationship was shown to exist between the protein in the diets and the appearance of deficiency signs (Wintrobe *et al.*, 1945). Only when the protein (casein) content was low (10%) was a niacin deficiency precipitated. This was reflected in decreased urinary excretion of the vitamin and its derivatives. Similar findings have been reported for the rat (Sarett, Klein, and Perlzweig, 1942). However, in the case of the dog, reduction in the protein content of the diet caused an increase in the urinary excretion of niacin and derivatives. Since the reverse (a decreased excretion on high protein diets) was also obtained, the authors concluded that in this species retention of niacin is associated with protein anabolism. During the catabolism resulting from starvation, the dog excreted increased quantities of niacin (Sarett, Klein, and Perlzweig, 1942).

An imbalance in amino acid intake, resulting from the inclusion of zein or other low-tryptophan proteins in the niacin deficient diet, can also depress rat growth (Krehl, Henderson *et al.*, 1946). Of the individual amino acids tested thus far, glycine has been shown to be most responsible. However, other amino acids may be involved, since similar results were obtained with acid-hydrolyzed casein and zein which contain little or no glycine. In these instances of amino acid imbalance, intestinal synthesis of the vitamin is also undoubtedly involved since the nature of the carbohydrate in the ration is a contributing factor. Only when sucrose replaced dextrin in the ration was growth inhibition noted (Krehl, Henderson *et al.*, 1946).

The multiplicity of factors influencing bacterial synthesis of niacin is demonstrated by the observation (Elvehjem, 1946) that the addition of dried milk or lactose to the basal ration of rats augmented the production of both niacin and folic acid; and the addition of butterfat to a synthetic milk ration induced greater synthesis of these vitamins than corn oil. Raising the intake of either of these vitamins resulted in increased formation of the other, whereas sulfa drugs inhibited the bacterial synthesis.

Evidence for the synthesis of niacin by the intestinal flora of man was presented by Ellinger and associates (Ellinger and Coulson, 1944; Ellinger and Benesch, 1945) who observed a drop in urinary excretion of N^1-methylnicotinamide after the administration of the bacterial inhibitors, sulfasuxidine and sulfaguanidine. Intestinal synthesis of niacin was also demonstrated by aerobic incubation of human feces obtained during a cecostomy; under anaerobic conditions however, cecal organisms destroyed two-thirds of the vitamin (Ellinger and Benesch, 1945; Benesch, 1945). This would seem to indicate that in the normal cecum there is an equilibrium between the synthetic and destructive processes which may play a causal role in deficiency disease.

Further studies in support of niacin synthesis in the human gut are those of Najjar and associates (Najjar *et al.*, 1946). On an intake of as little as 1.5–2.0 mg. of niacin, the test subjects excreted in the urine quantities of N^1-methylnicotinamide equivalent to that found after the ingestion of a normal ration furnishing 20–30 mg. of niacinamide.

Little recognition has been given to the occurrence of antivitamins in natural foods and their possible role in precipitating vitamin deficiencies. Recent studies by Woolley indicate that the pellagragenic nature of corn may be attributed in part to the presence of an antivitamin, presumably a structural analogue of niacin, which he has actually been able to concentrate from its natural source (Woolley, 1946b). It is of interest to recall in this connection that 3-acetylpyridine in the ration can precipitate the reversible signs of niacin deficiency in mice and dogs, but not in microbial species (Woolley, 1946a).

Vitamin-vitamin interrelationships involving niacin as one of the participants have also been reported. The fact that niacin and ascorbic acid can react with each other (Milhort, 1944) has been mentioned in an earlier section of this paper. Whether or not the reaction product is physiologically available has, as yet, not been demonstrated. The dog grows well on balanced rations furnishing only six of the vitamins of the B complex, thiamine, riboflavin, niacin, pyridoxine, pantothenic acid and choline (Krehl, Torbet *et al.*, 1946). Apparently the bacterial flora in the dog can make in ample quantities the other members of the B complex. This is not the case, however, when a vitamin deficiency is produced. Thus, folic acid administration has been shown to affect the response of the dog with black tongue to niacin dosage. Dogs on diets low in niacin developed a folic acid deficiency in addition to the black tongue syndrome. Only when folic acid was included in the ration could niacin deficiencies be produced repeatedly, and these responded to the single administration of niacin. In the absence of a dietary source of folic acid, the dog frequently failed to respond after the production of two or three deficiencies (Krehl, Torbet *et al.*, 1946).

The papers discussed in this section of the present review make it mandatory that in studies of the physiological availability of niacin (or amide) close adherence to suitable standardized conditions be maintained. It is quite conceivable that apparently unimportant variations in experimental conditions can result in two investigators obtaining different results.

e. Pantothenic Acid. Little is known of the relative physiological availability of pantothenic acid in bound *vs.* free state. The excretion of this vitamin after administration of the calcium salt to dogs has been studied by Silber (1945). An inverse relationship was found between the urinary output and the amount of food ingested with the vitamin. Fecal loss of unabsorbed pantothenic acid occurred when the food intake was large. However, when calcium pantothenate was injected parenterally some was found in the feces.

The non-availability to man of the pantothenic acid in live yeast has been reported by Parsons and associates (1942).

Evidence for the synthesis of pantothenic acid, as well as *p*-aminobenzoic acid, biotin and folic acid, in the human adult, is furnished by studies showing the total urinary and fecal excretion of these vitamins to exceed the dietary intake (Denko *et al.*, 1946).

The interrelationship among the vitamins in respect to their synthesis and utilization of pantothenic acid is indicated by the observation that, following suppression of pantothenic acid synthesis by feeding succinylsulfathiazole, the deficiency symptoms could not be corrected by adding pantothenic acid alone, even parenterally. However, administration of biotin and folic acid caused prompt restoration of growth under these conditions, indicating that their effect is at least partly related to the utilization of pantothenic acid (Wright and Welch, 1944).

It was reported by Spies and associates (1940) that excessive dosage with riboflavin induced a rise in the blood pantothenic acid level. In the laboratories of the present authors, however, it has been found that this is merely a transitory effect, the elevated blood and urine values returning to the predosage levels after 1–2 weeks despite continued administration of the extra riboflavin (Melnick *et al.*, 1942).

f. Vitamin B_6. When the yeast, *Saccharomyces carlsbergensis*, is employed for the determination of vitamin B_6 activity (Atkin *et al.*, 1943), the values include the quantities of pyridoxine, pyridoxal, pyridoxamine (Snell and Rannefeld, 1945), and a postulated acid-labile factor (Melnick *et al.*, 1945) which may be present. The decrease in vitamin B_6 activity of some biological materials when treated with strong acid (Melnick *et al.*, 1945) can be restored for certain microorganisms but not for others (Rubin *et al.*, 1947). Furthermore, only a small part of the original vitamin B_6 activity, based upon biological assays with the rat, is recovered when strong acid extracts are neutralized to pH 1.5–2.0 and autoclaved again (Rubin *et al.*, 1946). Thus, the availability of the acid-labile component of the vitamin B_6 group (Melnick *et al.*, 1945) or of the bound forms of vitamin B_6 (Rubin *et al.*, 1947) for the animal organism requires further investigation.

The composition of the ration has been found to have a profound effect upon the requirements for vitamin B_6. The addition of tryptophan reduces the period of survival of mice deficient in this vitamin but not to the same extent as an equivalent amount of tryptophan furnished in the form of casein (Miller and Baumann, 1945). From this it is apparent that amino acids other than tryptophan interfere with the health of vitamin B_6-deficient mice. Confirmatory data in studies with the dog (Morgan *et al.*, 1946) and with the rat (Sarma *et al.*, 1947) have been published. Methionine similarly had a growth depressing effect on vitamin B_6-deficient rats (Sarma *et al.*, 1947). On the other hand, the toxicity of tyrosine for the rat was greatly reduced when fed to pyridoxine-deficient animals (Martin, 1946).

Vitamin B_6 is also involved in the metabolism of unsaturated fatty acids. Both the vitamin and essential fatty acids have been shown to cure rat dermatitis (Birch, 1938; Schneider *et al.*, 1940; Salmon, 1941; Quackenbush *et al.*, 1942). The addition of oleic acid to a ration containing suboptimal amounts of pyridoxal or pyridoxine caused a growth inhibition, which was counteracted by increasing the vitamin intake (Sarma *et al.*, 1947).

Carbohydrate in the dietary can also affect the vitamin B_6 requirement by influencing the bacterial synthesis of the vitamin in the intestinal tract. Thus, when dextrin replaced sucrose in the vitamin-free diet, rats grew fairly well and showed no evidence of vitamin B_6 deficiency (Sarma *et al.*, 1946). Through measurements of pyridoxic acid excretion in the urine, it was demonstrated that synthesis and utilization of vitamin B_6-active compounds occurred (Sarma *et al.*, 1946).

Microorganisms in the intestinal tract may also destroy members of the vitamin B_6 group. Pyridoxal or pyridoxamine, when fed as a component of the ration, was partially destroyed during transit through the gastrointestinal tract. When these compounds were given parenterally or alone *per os* greater biological responses were obtained (Sarma *et al.*, 1946).

The above studies emphasize the need for highly standardized technics and adequate controls when assays are conducted for the physiological availability of vitamin B_6 using either the growth response of the rat or pyridoxic acid excretion in the case of the human as the basis for the evaluation.

g. Biotin. It is now well recognized that the etiological factor in egg white injury in the rat, chicken, and man, is the presence of avidin, a protein which combines with biotin so firmly as to make it unavailable for absorption (György *et al.*, 1941; Sydenstricker *et al.*, 1942) although the biotin is utilized when the combination is given parenterally (György and Rose, 1941). Since the biotin-avidin complex is labile to heat, the effect is observed only after the ingestion of large amounts of raw or slightly heated egg white. Crystalline avidin has 15,000 times the biotin-binding capacity of raw egg white, one molecule of avidin combining with one molecule of biotin (Woolley and Longsworth, 1942). A slight degree of biotin-combining capacity is also manifested by other proteins with a high isoelectric point, *e.g.*, salmine from salmon sperm and nucleohistone from liver (Woolley and Longsworth, 1942). Rancid fat tends to inactivate biotin but tocopherol inhibits this process (Pavcek and Shull, 1942).

Like other vitamins of the B group, the biotin of fresh yeast has been shown not to be available for absorption by man (Parsons *et al.*, 1942).

Though rats are able to thrive on a biotin-deficient diet, the inclusion of sulfaguanidine or succinylsulfathiazole in such diets results in the characteristic syndrome of biotin deficiency which can be corrected (or prevented) by the administration of the vitamin (Nielsen and Elvehjem, 1942; Nielsen *et al.*, 1942; Daft *et al.*, 1942). The degree of intestinal synthesis of this vitamin was higher on a stock diet than on diets low in pantothenic acid or riboflavin.

Adult women receiving diets of high, low or intermediate biotin content, excreted much more of this vitamin than they consumed, although relatively lower output occurred on the high-biotin diets (Gardner *et al.*, 1945). Oral administration of biotin methyl ester resulted in its rapid elimination in the urine. However, it was not established whether the biotin was excreted in the free form since all urine samples were acid-hydrolyzed prior to microbiological assay. If the biotin were in fact in the free form under these experimental conditions, the physiological availability to man of the esterified biotin would be indicated. Additional evidence of the intestinal synthesis of biotin, based upon excretion data, is furnished by the work of others (Oppel, 1942; Najjar and Holt, 1943; Najjar *et al.*, 1944; Denko *et al.*, 1946).

It has recently been found in pigs that the addition of inositol to a basal ration containing sulfathalidine alleviated the symptoms of biotin deficiency ordinarily induced by such a diet (Lindley and Cunha, 1946). Evidently the inositol stimulated intestinal synthesis of biotin to an extent sufficient to offset the inhibitory effect of the sulfa drug. Both biotin and folic acid were required to counteract the growth-inhibitory effect of succinylsulfathiazole in the diet (Elvehjem, 1946). When one of these vitamins was added to the ration, a deficiency in the other developed.

The studies, reviewed above, demonstrate that the protein component of the ration and intestinal synthesis of biotin should be standardized n studies of biotin availability. It is preferable that free biotin be employed as the reference standard since the availability to the human of the esterified forms has not been established.

h. p-Aminobenzoic Acid. Some evidence has been presented to indicate that the absorption of *p*-aminobenzoic acid is dependent upon the bile salts (Greaves and Schmidt, 1937).

Rats grow well on a diet containing thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine and choline. When both inositol and *p*-aminobenzoic acid are added, no improvement in growth results. However, on the addition of one of these factors, a need for the other develops (Martin, 1942a). Apparently under such circumstances intestinal synthesis of these vitamins is so altered that dietary sources are equired.

In experiments on man it was shown that the total urinary and fecal values for *p*-aminobenzoic acid exceeded dietary intake, demonstrating hat, in this species also, synthesis of this factor occurs (Denko, 1946).

p-Aminobenzoic acid is an essential nutrient for certain microorgansms. Since sulfanilamide is a structural analogue of *p*-aminobenzoic acid, it reacts competitively in the metabolism of the microorganism thus ausing bacteriostasis. This subject has been adequately treated in a eview by Woolley (1946a).

Since *p*-aminobenzoic acid is a unit in the structure of another vitamin, olic acid (pteroylglutamic acid), studies of the urinary excretion of the ormer should be included in investigations dealing with the metabolism f the latter vitamin. What influence this potential source of *p*-aminobenzoic acid has on studies designed to determine factors which modify he available supply of *p*-aminobenzoic acid remains to be elucidated.

i. Folic Acid. Following the establishment of the molecular constitution of folic or pteroylglutamic acid, the nature of several of the moleclar conjugates of this vitamin was demonstrated. The marked ifferences observed in physiological availability of these conjugates to arious species of animals or microorganisms had been responsible for the pparent multiplicity of factors now recognized as variants of pteroyllutamic acid. The conjugate known as fermentation *L. casei* factor which is just as active, mole for mole, as pteroylglutamic acid for *L. casei*, ats, chicks and monkeys, is relatively inactive for *Streptococcus faecalis* ; on the other hand, the degradation product pteroic acid, in which the lutamic acid portion of the pteroylglutamic acid molecule is replaced by hydrogen is active for the latter microorganism but not for *L. casei* (Jukes, 1946). In this connection it is of interest that xanthopterin was found to be active for cytopenic monkeys, but failed permanently to prevent the deficiency (Totter *et al.*, 1944).

The crystalline conjugate isolated from yeast is available to the chick but not to the bacteria employed for assay purposes. Since incubation of the fermentation *L. casei* factor with homogenized fresh chick liver in the presence of β-pyracin resulted in doubling the activity for *Strep. faecalis* R, Daniel and associates (1945) concluded that this form of folic acid participates with β-pyracin in an enzymic reaction, the end-product of which exhibits increased biological potency. This hypothesis has been questioned on the ground that both fermentation and liver *L. casei* factors are about equally active for the chick and the addition of β-pyracin is not necessary for growth, feathering or hematopoiesis in this species (Campbell *et al.*, 1946; Hutchings *et al.*, 1946). Since the liver factor has a lower molecular weight than the fermentation *L. casei* factor, it is more probable that the enzymic activation, if any, is a degradative process rather than a conjugation of β-pyracin with the fermentation factor.

The failure of rats to develop a characteristic deficiency syndrome when fed purified diets lacking folic acid (Mitchell *et al.*, 1941; Martin, 1942b; Nielsen and Elvehjem, 1942; Daft and Sebrell, 1943) is now regarded as due to bacterial synthesis of this factor in the intestinal tract. The incorporation of insoluble sulfonamides in such experimental rations inhibits folic acid production to such an extent that requirements for growth are no longer met. Supplemental dosage of crystalline folic acid or concentrate counteracts this effect.

As has already been pointed out in a previous section, intestinal synthesis in the rat of both folic acid and niacin was reported to be stimulated by the addition of lactose or milk powder (Elvehjem, 1946), or by the replacement of corn oil with butterfat. Excess niacin also increased folic acid production and *vice versa*. The poor growth resulting from the administration of succinylsulfathiazole was prevented by the incorporation of biotin and folic acid in the ration. If either vitamin was added alone, a deficiency in the other developed (Elvehjem, 1946).

Somewhat in contrast to their effect in rats, the sulfa drugs, and streptomycin as well, have been shown to enhance intestinal synthesis of folic acid in chickens (Moore *et al.*, 1946). This has been explained by a modification of the type of flora resulting in suppression of those organisms which either have a folic acid-destructive action or produce toxic substances interfering with normal metabolism. Dogs receiving a synthetic diet containing 6 of the B vitamins but excluding folic acid, grew well even when 4% sulfasuxidine was included (Krehl, Torbet *et al.*, 1946). However, during nicotinic acid deficiency the dog exhibited a requirement for dietary folic acid.

Experiments by Najjar *et al.* (1944; 1945) have demonstrated that man can subsist without a dietary source of folic acid. In these studies a synthetic diet was employed such as that used to induce the deficiency in rats, except that dextrimaltose was added and sulfonamides omitted. Though no clinical evidence of folic acid deficiency was observed in the test subjects despite 18 months on this dietary regime, it would be unsound to generalize that there is no need for folic acid in the human dietary, particularly in view of the susceptibility of the bacterial flora to change. Denko and associates (1946) have also demonstrated that the human under normal conditions is capable of synthesizing folic acid.

Whereas the folic acid derived from usual dietary sources and from intestinal synthesis suffice for normal individuals, the vitamin from such sources appears to be incompletely available to the pernicious anemia patient; on the other hand, the clinical effectiveness of synthetic folic acid in this condition, is quite dramatic (Bethell *et al.*, 1946). In urinary excretion studies Welch and associates (1946), and subsequently Bethell and collaborators, demonstrated that the conjugate present in yeast (pteroylheptaglutamic acid) was excreted largely as free folic acid in normal subjects, whereas in cases of pernicious anemia this conjugate, unlike free folic acid, was not effectively utilized nor did it give rise to increased urinary excretion of folic acid. The former authors (Welch *et al.*, 1946) suggest that the anti-pernicious anemia factor in liver extract may be a component of the conjugase system which hydrolyzes folic acid conjugates, or it may in some way offset the effect of naturally-occurring substances which inhibit such conjugase activity. If β-pyracin were the active factor in liver extract, administration of this compound to pernicious anemia patients receiving conjugated folic acid (*e.g.*, as found in yeast) plus dietary inhibitors of the conjugase reaction, should result in urinary excretion of free folic acid and clinical improvement. To the present authors' knowledge such studies have not as yet been conducted. That valuable information on folic acid availability can be obtained by the urinary excretion technic is demonstrated by the latter two studies cited.

j. Inositol. Though inositol has long been known to be a constituent of heart muscle and of phytin, its identity with bios I and its vitamin activity in the mouse and rat have only recently been demonstrated. Woolley has studied factors influencing intestinal synthesis of inositol in the mouse (Woolley, 1942). It is suggested by his work that the microorganisms responsible for inositol synthesis require pantothenic acid, since increased inositol formation resulted when that vitamin was added to the diet of mice. It is possible, however, that pantothenic acid may influence the intestinal absorption of inositol since a diet containing the latter but not pantothenic acid has been shown to produce the alopecia of inositol deficiency; an alternative explanation is that both of these nutrients may be involved in hair growth.

Evidence has previously been presented indicating that a relationship exists between inositol and *p*-aminobenzoic acid (Martin, 1942a). The simultaneous addition of both of these vitamins to a diet lacking these factors, but otherwise adequate, resulted in no more favorable growth; however, the addition of either alone precipitated signs of deficiency of the other. This observation has been explained by alteration of the process of intestinal synthesis.

Investigations with human subjects of the urinary excretion of inositol have been reported by Johnson and associates (1945). In view of the small excretion values (in the neighborhood of 0.5 mg./hour) relative to dietary intake,[3] it would seem that some other excretory product than free inositol would have to be determined in studies of physiological availability.

k. Choline. Choline availability is intimately linked with protein intake. The lipotropic activity of choline was demonstrated (Best and Lucas, 1943; Best and Ridout, 1940) by its efficacy in preventing or curing the fatty livers developed in rats on a low-protein, high-fat ration. Whereas methionine was also effective, cystine produced the opposite effect, *i.e.*, increased markedly the fatty infiltration of the liver. Because of these observations, it was concluded that the lipotropic activity of proteins was dependent in large part on the amounts and proportions of these sulfur-containing amino acids. In studies with young rats of the hemorrhagic degeneration of the kidney induced by feeding a choline-deficient diet, cures were also brought about by administration of choline and methionine, while cystine inhibited the activity of these compounds (Griffith and Wade, 1940; Griffith, 1941a). These studies have demonstrated that the dietary deficiency is concerned specifically with the available supply of labile methyl groups.

The determination of the level of a test material required to protect against fatty infiltration of the liver has been proposed as the basis of a biological procedure for the assay of choline (Griffith, 1941b). Weanling rats have been used for this purpose. This method actually measures the labile methyl groups furnished, since betaine, sarcosine and methionine are also effective. Thus, rats on a low choline diet, furnishing an adequate supply of methionine, have been found capable of synthesizing the vitamin (Jacobi *et al.*, 1941). Choline synthesis was reduced somewhat on a diet high in fat, but other changes in the ration, such as increasing the protein, cystine or choline were without marked effect on the choline content of the tissues. However, the need of the rat for the prevention of fatty livers and kidney degeneration suggests that this synthesis of choline occurs only at a limited rate.

---

[3] On the basis of food analyses, conducted in the present authors' laboratory and elsewhere, the urinary excretion of inositol is about 2% of the intake.

In the chick not all of the nutritional effects of choline are obtained by feeding methionine. Jukes (1941) demonstrated with this species that methionine could not replace the vitamin in curing perosis, although some of the functions of choline can be assumed by methionine and also by betaine (Almquist and Grau, 1944).

Whereas other vitamins have proved to be ineffective in curing the hemorrhagic degeneration of the kidney in the rat (Griffith and Mulford, 1941), inositol was found (Engel 1942) to reduce fatty infiltration of the liver. Nicotinic acid supplements, on the other hand, were noted to exert a moderate choline-opposing action (Griffith and Mulford, 1941). The latter effect may be attributed to diversion of labile methyl groups to the formation of N^1-methylnicotinamide, which is then rapidly excreted in the urine.

The studies with choline demonstrate that the quantity and composition of the protein in the test ration should be controlled in physiological availability studies based upon urinary excretion responses. It is not known at the present time in what forms the extra labile methyl groups are excreted in the urine. These may vary depending upon the uncontrollable dietary factors, *viz.*, nicotinamide intake. Measurement of the urinary excretion of choline, as such, does not seem to afford a promising approach to the problem, since only very small amounts (less than 1%) are excreted in the urine following dosage (Johnson *et al.*, 1945a). The fecal excretion values are even lower.

V. UNSOLVED PROBLEMS OF PHYSIOLOGICAL AVAILABILITY IN HUMAN NUTRITION

With the physiological availability technic presented in this report it should be possible to assess not only whether vitamins under certain conditions are available for absorption, but also to determine the influence of those factors which can be responsible for increasing or decreasing the biological response following the ingestion of a given amount of the vitamin. Examples of such problems are presented in this section of the report.

1. *Conditioned Malnutrition*

Conditioned malnutrition has been defined by Jolliffe (1943) as a state of vitamin deficiency in individuals subsisting on normal diets but failing to absorb or utilize adequately the vitamins consumed. That the urinary excretion technic has proved to be of great value in assessing whether or not a dietary intake, satisfactory for normal individuals, meets the requirements of individuals in a pathological state is apparent from the many papers cited by Jolliffe (1943). It should suffice in studying a given condition, where an augmented requirement seems indicated, to furnish increasing quantities of the factor until a dose is arrived at which elicits a urinary response within the range observed in normal subjects. As a guide in this direction reference might be made to Table V, which presents the urinary excretion values obtained with the five test subjects routinely employed in our own physiological availability studies (Melnick *et al.*, 1945a). It is recognized that the excretion figures listed in Table V are all well within the normal range so that an estimate of requirements based upon these as the reference standard would be on the liberal side.

TABLE V

Urinary Excretion of Vitamins (or Derivatives) by Test Subjects

(All values in mg.)

Subject	Surface area in sq. m.	Excretion[1] of vitamin or derivative, per 24 hrs.							
		Thiamine		Ascorbic acid		Riboflavin		N^1-methylnicotinamide[3]	
		Basal	Increment after test dose[2]	Basal	Increment after test dose[2]	Basal	Increment after test dose[2]	Basal	Increment after test dose[2]
J. C.	1.70	0.33	1.03	18	121	0.73	4.26	8.0	14.7
E. M.	1.76	0.42	0.62	18	101	0.78	4.50	7.2	13.6
D. M.	1.88	0.39	0.93	36	117	0.57	4.84	7.7	9.8
M. H.	1.96	0.27	1.04	60	113	0.71	4.65	4.8	5.2
H. H.	2.15	0.31	0.84	52	121	0.74	4.70	3.8	7.7
Average	1.89	0.34	0.89	37	115	0.71	4.59	6.3	10.2

[1] On the day that urine samples were collected, each subject consumed the diet shown in Table II.

[2] The test dose of 5 mg. thiamine, 200 mg. ascorbic acid, 10 mg. riboflavin and 50 mg. nicotinamide in pure solution was taken orally immediately after the mid-day meal.

[3] Results expressed as nicotinamide on an equimolar basis.

2. Biosynthesis of Vitamins

The foregoing review has indicated the frequency with which urinary excretion studies are employed in attempts to evaluate whether the vitamins synthesized by the bacterial flora in the gastrointestinal tract of man are nutritionally available. The problem arising from the widespread use of the sulfonamides and its effect on the bacterial synthesis of known and as yet unknown factors can be studied quantitatively with the urinary excretion technic. The length of time that such drugs are ordinarily used by a patient is usually too short to allow the development of a deficiency condition although the malaise frequently encountered as a sequel to sulfa therapy may be related in part to inhibition of intestinal synthesis. As prophylactic agents to a number of diseases these compounds are used for longer periods of time. The possibility of inhibiting synthesis of vitamins upon which the organism depends in part for its requirements during such periods of medication should not be ignored. The urinary excretion of the vitamin would serve as a useful guide to indicate whether or not the tissue stores of these factors within the individual are being depleted during the period of sulfonamide administration.

3. Vitamin-Vitamin and Vitamin-Amino Acid Interrelationships

Vitamin-vitamin and vitamin-amino acid interrelationships have been shown in the preceding sections of this report to play a significant role in determining the available vitamin content under a given set of conditions. Most of the studies cited have been conducted on experimental animals. Can a vitamin imbalance be produced in the human by overdosing with only certain of the vitamins as suggested by Morgan (1941)? This problem could be approached through the urinary excretion technic. If overdosage with certain vitamins is responsible for excessive excretion (or greater destruction) of another vitamin, the change in the urinary excretion picture of this other factor should be halted on cessation of the overdosage; on administration of a test dose, evidence of depleted stores of the vitamin thrown into imbalance would be obtained through the finding that only a small fraction of the dose was recovered in the urine.

The role of tryptophan in modifying the requirement of the rat for niacin has been mentioned in an earlier section of this report. Whether the same relationship holds for the human could very easily be discovered by duplicating the studies of Rosen and associates on the rat (Rosen *et al.*, 1946). An increased intake of tryptophan should result in an increased excretion in the urine of N^1-methylnicotinamide, provided that the human has the ability to synthesize nicotinamide.

On the other hand, if an amino acid such as tyrosine taken in large doses can be responsible for precipitating an ascorbic acid deficiency (Sealock, 1945), such a phenomenon could also be investigated by the urinary excretion technic. The normal subjects on a constant basal intake containing extra quantities of tyrosine might be expected to show a modification in the urinary output of ascorbic acid. This may be an increase (due to a wastage of the vitamin by way of urinary excretion) or a decrease due to greater destruction of the vitamin within the organism. In any case, the urinary excretion picture on cessation of the tyrosine dosage, particularly after giving an oral test dose of ascorbic acid, should indicate whether or not depletion of the ascorbic acid stores in the individual was effected as a result of taking this amino acid in large dosage.

4. Antivitamins

The current hypothesis for the competitive antagonism between structural analogues of vitamins and the metabolites is that the two compounds compete for space on a portion of the enzyme participating in a particular metabolic reaction. If the vitamin combines with the enzyme group the compound formed passes through the normal metabolic reaction for which the system is adapted. If the analogue of the vitamin combines with the enzyme group, the resulting compound fails to fit into the biological scheme and therefore is of no value to the organism. Woolley (1946a) points out that while this hypothesis seems logical, there is little experimental proof that the vitamin analogues really do displace vitamin (presumably as a coenzyme) from protein combinations. If this concept were true, one would expect, in conducting urinary excretion studies with humans, that the administration of an antivitamin should be followed by an increased urinary output of the vitamin in question. On terminating this regime and following this immediately with a test dose of the vitamin, one should now expect the vitamin to replace its analogue from the protein combination resulting in only a small urinary excretion of the test dose. Of course the assay technic selected for determining the urinary excretion values should measure only the vitamin and not its analogue.

Studies along the lines suggested need not be carried to a point where a vitamin deficiency is induced by the administration of the antivitamin. Measurements of the urinary excretion of the water-soluble vitamins immediately reflect variations in vitamin intake provided normal subjects are employed (Melnick *et al.*, 1939). This feature of the assay technic may, therefore, be effectively utilized in the procedure described above for determining the mechanism of action of the antivitamins, without anticipated risk to the test subjects.

5. Availability of the Water-Insoluble Forms of the Water-Soluble Vitamins

The Food and Nutrition Board of the National Research Council (Gunderson, 1943) has emphasized the need for water-insoluble forms of thiamine, niacin and riboflavin for the enrichment of cereal products which are rinsed either before or after cooking. The water-soluble vitamins previously sprayed on the exterior surfaces of particles are removed, thus obviating the standard method of enrichment. This has led to the synthesis of water-insoluble forms of the water-soluble vitamins (Badgett *et al.*, 1945; Huber *et al.*, 1946) and to the use of physical methods involving coating of the vitamins with a water-insoluble membrane (Furter *et al.*, 1946). If it can be demonstrated, following dosage with such a modified nutrient, that the form in which the vitamin is excreted in the urine is the same as when a pure aqueous solution of the vitamin is taken, and that similar quantities are excreted, it may be concluded that the modified vitamin is physiologically available to the human. Studies along these lines have already been completed by one group of investigators (Furter *et al.*, 1946).

As a corollary to the above, mention may be made of the adaptability of the human bioassay technic to the determination of the physiological availability of the water-soluble forms of riboflavin which itself is quite insoluble (Furter *et al.*, 1945). Fluorometric, microbiological and rat assays fail to yield the same values for riboflavin succinate. The compounds exhibit the same fluorescence as free riboflavin, poor microbiological responses, and biological values intermediate between these two. It would seem safe to conclude that these compounds are available to the human if it were found in urinary excretion studies that the same quantity of riboflavin was excreted in the urine as that following dosage with free riboflavin, and that the form in which the vitamin is excreted, as determined by fluorometric and microbiological assay, was the same both in control and test urine.

6. *Availability of the Niacin Derivative in Cereal Products*

Andrews (1945) has justifiably called attention to the question of the availability of the niacin derivative in cereal products which is unavailable to *L. arabinosis* until the material is subjected to strong acid or strong alkaline hydrolysis (Melnick, 1942). Evidence has been presented (Krehl *et al.*, 1944) that the dog can utilize this niacin derivative, whereas the chick apparently cannot. Despite the fact that the rationale of bread enrichment is based in part upon the vitamin content of whole wheat flour prior to processing and that the niacin derivative is a major component of the total niacin content of whole wheat, no information has as yet been obtained to indicate whether or not this natural compound is available to man. The assumption that it is equivalent to niacinamide in biological potency has, as yet, no experimental support. Inasmuch as concentrates of this compound can be isolated from extracts of wheat bran and other cereal products (Krehl and Strong, 1944) it should be possible to assay this material by the human physiological technic described in this report to determine whether or not this derivative is converted to niacinamide *in vivo.* If this were the case, the extra excretion of N^1-methylniacinamide following dosage with this niacin derivative should be equivalent to that obtained when a comparable dose of vitamin is administered. Consideration should be given to the possibility that the niacin derivative may be handled like niacin rather than niacinamide. The urinary excretions following dosage with these two forms of the vitamin differ both qualitatively and quantitatively (Hochberg *et al.*, 1945a; Johnson *et al.*, 1945b; Melnick *et al.*, 1940; 188; Sarett, Huff, and Perlzweig, 1942). Another complicating factor is that the free vitamin may be present in whole wheat as both niacin and niacinamide, predominantly as the acid (Krehl *et al.*, 1945b).

7. *Influence of Treating Foods with Various Salts During Processing*

The lability of thiamine in the presence of sulfite and nitrite is well known (Williams, 1939). Nevertheless, sulfites and nitrites are applied extensively in the food industry. In the course of dehydrating foods and during subsequent storage, it has been found very effective to pretreat the material with sulfite, thereby inhibiting the so-called "browning" reaction between sugar and amino acid which affects the taste and appearance as well as the color of many foods. During the war, extensive pretreatment of vegetables, particularly during the blanching process, was carried out prior to their dehydration. In this way, the shelf-life of the final products was greatly extended. The successful use of sulfite in this industry has led food technologists in the frozen food field to advocate the introduction of sulfite into the blanching fluid in the course of preparing the foods prior to deep freezing. Similarly, nitrites are used extensively in the curing of meats. Despite these practices, no information has been presented indicating whether or not the inclusion of sulfite or nitrite in the human dietary *via* the foods processed with salts of these anions interferes with the availability of thiamine furnished by other items in the ration. This problem could readily be studied using essentially the technic employed in determining the influence of the dietary thiaminase in clams on the availability of thiamine (Melnick *et al.*, 1945c).

VI. Concluding Remarks

In the present review evidence has been presented to show that the results of non-biological procedures of vitamin assay do not necessarily reflect available vitamin content. Such methods of analysis are of value in determining total vitamin content; they fail to demonstrate the inter-reactions among the dietary components during digestion or of the influence of the composition of the ration upon vitamin synthesis *in vivo*. Vitamin content and vitamin availability are not synonymous and, with respect to the latter, species differences are known to exist.

REFERENCES

1. Adlersberg, D., and Sobotka, H. 1943. *J. Nutrition* **25**, 255.
2. Ahmad, B., and Malik, K. S. 1933. *Indian J. Med. Research* **20**, 1033.
3. Alexander, B., and Landwehr, G. 1945. *Science* **101**, 229.
4. Almquist, H. J., and Grau, C. R. 1944. *J. Nutrition* **27**, 263.
5. Almquist, H. J., and Stokstad, E. L. R. 1936. *J. Nutrition* **12**, 329.
6. Andrews, J. S. 1945. *Trans. Am. Assoc. Cereal Chem.* **4**, 1.
7. Andrews, J. S., Boyd, H. M., and Terry, D. E. 1942. *Ind. Eng. Chem., Anal. Ed.* **14**, 271.
8. Arnold, A., and Elvehjem, C. A. 1939. *Am. J. Physiol.* **126**, 289.
9. Association of Official Agricultural Chemists, Washington, D. C., Official and Tentative Methods of Analysis. 1945.
10. Atkin, L., Schultz, A. S., Williams, W. I., and Frey, C. N. 1943. *Ind. Eng. Chem., Anal. Ed.* **15**, 141.
11. Atkin, L., Williams, W. I., Schultz, A. S., and Frey, C. N. 1944. *Ind. Eng. Chem., Anal. Ed.* **16**, 67.
12. Badgett, C. O., Provost, R. C., Jr., Ogg, C. L., and Woodward, C. F. 1945. *J. Am. Chem. Soc.* **67**, 1135.
13. Basinski, D. H., and Sealock, R. R. 1946. *J. Biol. Chem.* **166**, 7.
14. Bauernfeind, J. C., Sotier, A. L., and Boruff, C. S. 1942. *Ind. Eng. Chem., Anal. Ed.* **14**, 666.
15. Baxter, J. G., Robeson, C. D., Taylor, J. D., and Lehman, R. W. 1943. *J. Am. Chem. Soc.* **65**, 918.
16. Benesch, R. 1945. *Lancet* **I**, 718.
17. Bessey, O., and King, C. G. 1933. *J. Biol. Chem.* **103**, 687.
18. Best, C. H., and Lucas, C. C. 1943. *Vitamins & Hormones* **1**, 1.
19. Best, C. H., and Ridout, J. H. 1940. *J. Physiol.* **97**, 489.
20. Bethell, F. H., Swendseid, M. E., Bird, O. D., Meyers, M. C., Andrews, G. A., and Brown, R. A. 1946. *Univ. Hosp. Bull., Univ. Mich.* **12**, 42.
21. Bhagvat, K., and Devi, P. 1944. *Indian J. Med. Research* **32**, 131.
22. Birch, T. W. 1938. *J. Biol. Chem.* **124**, 775.
23. Bird, O. D., Robbins, M., Vandenbelt, J. M., and Pfiffner, J. J. 1946. *J. Biol. Chem.* **163**, 649.
24. Black, S., Overman, R. S., Elvehjem, C. A., and Link, K. P. 1942. *J. Biol. Chem.* **145**, 137.
25. Bliss, C. I., and Marks, H. P. 1939. *Quart. J. Pharm. Pharmacol.* **12**, 182.
26. Booth, R. G., Henry, K. M., and Kon, S. K. 1942. *Biochem. J.* **36**, 445.
27. Borsook, H., Davenport, H. W., Jeffreys, C. E. P., and Warner, R. C. 1937. *J. Biol. Chem.* **117**, 237.
28. Brenner, J. 1943. *Pharm. Acta Helv.* **18**, 283.
29. Brew, W., and Scott, M. B. 1946. *Ind. Eng. Chem., Anal. Ed.* **18**, 46.
30. Burkholder, P. R., McVeigh, I., and Wilson, K. 1945. *Arch. Biochem.* **7**, 287.
31. Cahill, W. M. 1941. *J. Nutrition* **21**, 411.

32. Campbell, C. J., Brown, R. A., Bird, O. D., and Emmett, A. D. 1946 *J. Nutrition* **32**, 423.
33. Clausen, S. W. 1938. *J. Am. Med. Assoc.* **111**, 144.
34. Clausen, S. W., McCoord, A. B., and Goff, B. L. 1946. *Federation Proc.* **5**, 129.
35. Clayton, M. M., and Borden, R. A. 1943. *J. Nutrition* **25**, 349.
36. Clayton, M. M., and Folsom, M. T. 1940. *J. Home Econ.* **32**, 390.
37. Cornbleet, T., Kirch, E. R., and Bergeim, O. 1943. *J. Am. Med. Assoc.* **122**, 426.
38. Crampton, E. W., and Burton, B. W. 1946. *Federation Proc.* **5**, 230.
39. Crook, E. M. 1941. *Biochem. J.* **35**, 226.
40. Crook, E. M., and Hopkins, F. G. 1938. *Biochem. J.* **32**, 1356.
41. Cross, M. C. A., Embleton, J., and Coward, K. H. 1946. *Biochem. J.* **40**, 458.
42. Curtis, A. C., and Kline, E. M. 1939. *Arch. Internal Med.* **63**, 54.
43. Czaczkes, J. W., and Guggenheim, K. 1946. *J. Biol. Chem.* **162**, 267.
44. Daft, F. S., Ashburn, L. L., and Sebrell, W. H. 1942. *Science* **96**, 321.
45. Daft, F. S., Endicott, K. M., Ashburn, L. L., and Sebrell, W. H. 1943. *Proc. Soc. Exptl. Biol. Med.* **53**, 130.
46. Daft, F. S., and Sebrell, W. H. 1943. *U. S. Pub. Health Service, Pub. Health Repts.* **58**, 1542.
47. Daniel, L. J., Scott, M. L., Norris, L. C., and Hauser, G. F. 1945. *J. Biol. Chem.* **160**, 265.
48. Dann, W. J. 1941. *J. Biol. Chem.* **141**, 803.
49. Davis, M. V., Oldham, H. G., and Roberts, L. J. 1946. *J. Nutrition* **32**, 143.
50. Denko, C. W., Grundy, W. E., Porter, J. W., Berryman, G. H., Friedemann, T. E., and Youmans, J. B. 1946. *Arch. Biochem.* **10**, 33.
51. Deuel, H. J., Jr., Sumner, E., Johnston, C., Polgár, A., and Zechmeister, L. 1945. *Arch. Biochem.* **6**, 157.
52. Deutsch, H. F., and Hasler, A. D. 1943. *Proc. Soc. Exptl. Biol. Med.* **53**, 63.
53. DeWitt, J. B., and Sullivan, M. X. 1946. *Ind. Eng. Chem., Anal. Ed.* **18**, 117.
54. du Vigneaud, V., Dittmer, K., Hague, E., and Long, B. 1942. *Science* **96**, 186.
55. Einhauser, M. 1936. *Z. ges. exptl. Med.* **98**, 461.
56. Ellinger, P., and Benesch, R. 1945. *Lancet* **48**, 432.
57. Ellinger, P., and Coulson, R. A. 1944. *Nature* **154**, 270.
58. Elmby, A., and Warburg, E. 1937. *Lancet* **II**, 1363.
59. Elvehjem, C. A. 1946. *J. Am. Dietet. Assoc.* **22**, 959.
60. Engel, R. W. 1942. *J. Nutrition* **24**, 175.
61. Evans, H. M., and Lepkovsky, S. 1929. *J. Biol. Chem.* **83**, 269.
62. Ewing, D. T., Kingsley, G. V., Brown, R. A., and Emmett, A. D. 1943. *Ind. Eng. Chem., Anal. Ed.* **15**, 301.
63. Federal Surplus Commodities Corp., Form SCP-1738, Program GCP. 1944.
64. Field, H., Jr., Robinson, W. D., and Melnick, D. 1940. *Ann. Internal Med.* **14**, 588.
65. Fischer, G. S. 1945. *Ind. Eng. Chem., Anal. Ed.* **17**, 224.
66. Fraps, G. S. 1946. *Arch. Biochem.* **10**, 485.
67. Fraps, G. S., and Meinke, W. W. 1945. *Food Research* **10**, 187.
68. Furter, M. F., Haas, G. J., and Rubin, S. H. 1945. *J. Biol. Chem.* **160**, 293.
69. Furter, M. F., Lauter, W. M., DeRitter, E., and Rubin, S. H. 1946. *Ind. Eng. Chem.* **38**, 486.
70. Gardner, J., Parsons, H. T., and Peterson, W. H. 1945. *Arch. Biochem.* **8**, 339.

71. Golumbic, C., and Mattill, H. A. 1941. *J. Am. Chem. Soc.* **63,** 1279.
72. Goss, H. 1943. *Nutrition Abstracts & Revs.* **12,** 531.
73. Gottlieb, H. F., Quackenbush, F. W., and Steenbock, H. 1943. *J. Nutrition* **25,** 433.
74. Greaves, J. D. 1939. *Am. J. Physiol.* **125,** 429.
75. Greaves, J. D., and Schmidt, C. L. A. 1933. *J. Biol. Chem.* **102,** 101.
76. Greaves, J. D., and Schmidt, C. L. A. 1934. *Univ. Calif. Pub. Physiol.* **8,** 43.
77. Greaves, J. D., and Schmidt, C. L. A. 1935. *Am. J. Physiol.* **111,** 492.
78. Greaves, J. D., and Schmidt, C. L. A. 1937. *Proc. Soc. Exptl. Biol. Med.* **37,** 40.
79. Green, R. G., Carlson, W. E., and Evans, C. A. 1942. *J. Nutrition* **23,** 165.
80. Griffith, W. H. 1935. *J. Nutrition* **10,** 667.
81. Griffith, W. H. 1941a. *J. Nutrition* **21,** 291.
82. Griffith, W. H. 1941b. *J. Nutrition* **22,** 239
83. Griffith, W. H., and Mulford, D. J. 1941. *J. Nutrition* **21,** 633.
84. Griffith, W. H., and Wade, N. J. 1940. *J. Biol. Chem.* **132,** 627.
85. Guerrant, N. B., and Dutcher, R. A. 1934a. *J. Nutrition* **8,** 397.
86. Guerrant, N. B., and Dutcher, R. A. 1934b. *Proc. Soc. Exptl. Biol. Med.* **31,** 796.
87. Guggenheim, K. 1944. *Biochem. J.* **38,** 260.
88. Gunderson, F. L. 1943. *Science* **98,** 277.
89. György, P., Rose, C. S., Eakin, R. E., Snell, E. E., and Williams, R. J. 1941. *Science* **93,** 477.
89a. György, P., and Rose, C. S. 1941. *Science* **94,** 261.
90. Harris, P. L., Jensen, J. L., Joffe, M., and Mason, K. E. 1944. *J. Biol.Chem.* **156,** 491.
91. Hart, E. B. 1945. *Nutrition Revs.* **3,** 33.
92. Hartzler, E. R. 1945. *J. Nutrition* **30,** 355.
93. Hathaway, M. L., and Lobb, D. E. 1946. *J. Nutrition* **32,** 9.
94. Hathaway, M. L., and Strom, J. E. 1946. *J. Nutrition* **32,** 1.
95. Hawley, E. E., Stephens, D. J., and Anderson, G. 1936. *J. Nutrition* **11,** 135.
96. Hickman, K. C. D., and Harris, P. L. 1946. *Advances in Enzymol.* **6,** 469.
97. Himwich, H. E., Goldfarb, W., and Cowgill, G. R. 1931–32. *Am. J. Physiol.* **99,** 689.
98. Hines, L. R., and Mattill, H. A. 1943. *J. Biol. Chem.* **149,** 549.
99. Hochberg, M., Melnick, D., and Oser, B. L. 1943a. *Ind. Eng. Chem., Anal. Ed.* **15,** 182.
100. Hochberg, M., Melnick, D., and Oser, B. L. 1943b. Unpublished studies.
101. Hochberg, M., Melnick, D., and Oser, B. L. 1945a. *J. Biol. Chem.* **158,** 265.
102. Hochberg, M., Melnick, D., and Oser, B. L. 1945b. *J. Nutrition* **30,** 193.
103. Hochberg, M., Melnick, D., and Oser, B. L. 1945c. *J. Nutrition* **30,** 201.
104. Hochberg, M., Melnick, D., and Oser, B. L. 1945d. *J. Nutrition* **30,** 225.
105. Hodson, A. Z., and Norris, L. C. 1939. *J. Biol. Chem.* **131,** 621.
106. Hopkins, F. G., and Morgan, E. J. 1936. *Biochem. J.* **30,** 1446.
107. Horowitz, N. H., and Beadle, G. W. 1943. *J. Biol. Chem.* **150,** 325.
108. Horwitt, M. K., Cowgill, G. R., and Mendel, L. B. 1936a. *J. Nutrition* **12,** 237.
109. Horwitt, M. K., Cowgill, G. R., and Mendel, L. B. 1936b. *J. Nutrition* **12,** 255.
110. Huber, W., Boehme, W., and Laskowski, S. C. 1946. *J. Am. Chem. Soc.* **68,** 187.

111. Hutchings, B. L., Oleson, J. J., and Stokstad, E. L. R. 1946. *J. Biol. Chem.* **163,** 447.
112. Jacobi, H. P., Baumann, C. A., and Meek, W. J. 1941. *J. Biol. Chem.* **138,** 571.
113. *J. Am. Med. Assoc.* **131,** 1426. 1946. Current Comment.
114. Joffe, M., and Harris, P. L. 1943. *J. Am. Chem. Soc.* **65,** 925.
115. Johnson, B. C., Hamilton, T. S., and Mitchell, H. H. 1945a. *J. Biol. Chem.* **159,** 5.
116. Johnson, B. C., Hamilton, T. S., and Mitchell, H. H. 1945b. *J. Biol. Chem.* **159,** 231.
117. Johnson, B. C., Hamilton, T. S., and Mitchell, H. H. 1945c. *J. Biol. Chem.* **159,** 425.
118. Johnson, B. C., Mitchell, H. H., and Hamilton, T. S. 1945d. *J. Biol. Chem.* **161,** 357.
119. Johnson, B. C., Hamilton, T. S., and Mitchell, H. H. 1945e. *J. Biol. Chem.* **158,** 619.
120. Jolliffe, N., Handbook of Nutrition. 1943. Am. Med. Assoc., 521.
121. Jukes, T. H. 1941. *J. Nutrition* **22,** 315.
121a. Jukes, T. H. 1946. Personal communication.
122. Jurist, V., and Foy, T. R. 1944. *J. Bact.* **47,** 434.
123. Karrer, P., and Straus, W. 1938. *Helv. Chim. Acta* **21,** 1624.
124. Keresztesy, J. C., and Sampson, W. L. 1937. *Proc. Soc. Exptl. Biol. Med.* **36,** 686.
125. Kertesz, Z. I. 1938. *Biochem. J.* **32,** 621.
126. Kirch, E. R., Cornbleet, T., and Bergeim, O. 1943. *Proc. Soc. Exptl. Biol. Med.* **54,** 307.
127. Kline, O. L. 1946. Estimation of the Vitamins, *Biol. Symposia* **13.** Catell Press, Lancaster, Pa.; Editors, W. J. Dann and G. H. Satterfield.
128. Klopp, C. T., Abels, J. C., and Rhoads, C. P. 1943. *Am. J. Med. Sci.* **205,** 852.
129. Knox, W. E., and Grossman, W. I. 1946. *J. Biol. Chem.* **166,** 391.
130. Krampitz, L. O., and Woolley, D. W. 1944. *J. Biol. Chem.* **152,** 9.
131. Krehl, W. A., de la Huerga, J., and Elvehjem, C. A. 1946. *J. Biol. Chem.* **164,** 551.
132. Krehl, W. A., de la Huerga, J., Elvehjem, C. A., and Hart, E. B. 1946. *J. Biol. Chem.* **166,** 53.
133. Krehl, W. A., Elvehjem, C. A., and Strong, F. M. 1944. *J. Biol. Chem.* **156,** 13.
134. Krehl, W. A., Henderson, L. M., de la Huerga, J., and Elvehjem, C. A. 1946. *J. Biol. Chem.* **166,** 531.
135. Krehl, W. A., Sarma, P. S., Teply, L. J., and Elvehjem, C. A. 1946. *J. Nutrition* **31,** 85.
136. Krehl, W. A., and Strong, F. M. 1944. *J. Biol. Chem.* **156,** 1.
137. Krehl, W. A., Teply, L. J., and Elvehjem, C. A. 1945a. *Science* **101,** 283.
138. Krehl, W. A., Teply, L. J., Sarma, P. S., and Elvehjem, C. A. 1945b. *Science* **101,** 489.
139. Krehl, W. A., Torbet, N., de la Huerga, J., and Elvehjem, C. A. 1946. *Arch. Biochem.* **11,** 363.
140. Kreula, M., and Virtanen, A. I. 1939. *Upsala Läkarefören. Förh.* **45,** 355.
141. Kringstad, H., and Lie, J. 1942. *Tids. Kjemi Bergvesen* **2,** 57; 1944. *Chem. Abstracts* **38,** 2367.

142. Lease, E. J., Lease, J. G., Steenbock, H., and Baumann, C. A. 1939. *J. Nutrition* **17**, 91.
143. Light, A. E., and Clarke, M. F. 1943. *J. Biol. Chem.* **147**, 739.
144. Light, R. F., Schultz, A. S., Atkin, L., and Cracas, L. J. 1938. *J. Nutrition* **16**, 333.
145. Lindley, D. C., and Cunha, T. J. 1946. *J. Nutrition* **32**, 47.
146. Lugg, J. W. H. 1942. *Nature* **150**, 577.
147. Mannering, G. J., Orsini, D., and Elvehjem, C. A. 1944. *J. Nutrition* **28**, 141.
148. Martin, G. J. 1942a. *Am. J. Physiol.* **136**, 124.
149. Martin, G. J 1942b. *Proc. Soc. Exptl. Biol. Med.* **51**, 353.
150. Martin, G. J. 1946. *J. Biol. Chem.* **166**, 389.
151. May, C. D., and McCreary, J. F. 1940. *J. Pediatrics* **17**, 143.
152. McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A. 1921. *Bull. Johns Hopkins Hosp.* **33**, 31.
153. McIntire, J. M., Henderson, L. M., Schweigert, B. S., and Elvehjem, C. A. 1943. *Proc. Soc. Exptl. Biol. Med.* **54**, 98.
154. McMillan, T. J., and Todhunter, E. H. 1946. *Science* **103**, 196.
155. Melnick, D. 1942. *Cereal Chem.* **19**, 553.
156. Melnick, D., Hochberg, M., Himes, H. W., and Oser, B. L. 1945a. *J. Biol. Chem.* **160**, 1.
157. Melnick, D., Hochberg, M., and Oser, B. L. 1942. Unpublished studies.
158. Melnick, D., Hochberg, M., and Oser, B. L. 1945a. *J. Nutrition* **30**, 67.
159. Melnick, D., Hochberg, M., and Oser, B. L. 1945b. *J. Nutrition* **30**, 81.
160. Melnick, D., Hochberg, M., and Oser, B. L. 1945c. *J. Nutrition* **30**, 233.
161. Melnick, D., Robinson, W. D., and Field, H., Jr. 1939. *J. Nutrition* **18**, 593.
162. Melnick, D., Robinson, W. D., and Field, H., Jr. 1940. *J. Biol. Chem.* **136**, 145.
163. Melnick, D., Robinson, W. D., and Field, H., Jr. 1941. *J. Biol. Chem.* **138**, 49.
164. Meyer, F. L., and Hathaway, M. L. 1944. *J. Nutrition* **28**, 93.
165. Mickelsen, O., and Keys, A. 1943. *J. Biol. Chem.* **149**, 479.
166. Milhort, T. H. 1944. *Proc. Soc. Exptl., Biol. Med.* **55**, 52.
167. Miller, E. C., and Baumann, C. A. 1945. *J. Biol. Chem.* **157**, 551.
168. Mitchell, H. H. 1943. *Vitamins & Hormones* **1**, 157.
169. Mitchell, H. K., and Isbell, E. R. 1942. *Univ. Texas Publ. No.* **4237**, 125.
170. Mitchell, H. K., Snell, E. E., and Williams, R. J. 1941. *J. Am. Chem. Soc.* **63**, 2284.
171. Moore, P. R., Evenson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., and Hart, E. B. 1946. *J. Biol. Chem.* **165**, 437.
172. Moore, T. 1945. *Vitamins & Hormones* **3**, 1.
173. Morgan, A. F. 1941. *Science* **93**, 261.
174. Morgan, A. F., Cook, B. B., and Davison, H. G. 1938. *J. Nutrition* **15**, 27.
175. Morgan, A. F., Groody, M., and Axelrod, H. E. 1946. *Am. J. Physiol.* **146**, 723.
176. Muelder, K. D., and Kelly, E. 1942. *J. Nutrition* **23**, 335.
177. Musulin, R. R., and King, C. G. 1936. *J. Biol. Chem.* **116**, 409.
178. Najjar, V. A., and Barrett, R. 1945. *Vitamins & Hormones* **3**, 23.
179. Najjar, V. A., and Holt, L. E., Jr. 1943. *J. Am. Med. Assoc.* **123**, 683.
180. Najjar, V. A., Holt, L. E., Jr., Johns, G. A., Mediary, G. C., and Fleischmann, G. 1946. *Proc. Soc. Exptl. Biol. Med.* **61**, 371.
181. Najjar, V. A., Johns, G. A., Mediary, G. C., Fleischmann, G., and Holt, L. E., Jr. 1944. *J. Am. Med. Assoc.* **126**, 357.

182. Neal, A. L., and Strong, F. M. 1943. *Ind. Eng. Chem., Anal. Ed.* **15,** 654.
183. Ness, H. T., Price, E. L., and Parsons, H. T. 1946. *Science* **103,** 198.
184. Nielsen, E., and Elvehjem, C. A. 1942. *J. Biol. Chem.* **145,** 713.
185. Nielsen, E., Shull, G. M., and Peterson, W. H. 1942. *J. Nutrition* **24,** 523.
186. *Nutrition Revs.* **1,** 214 (1943).
187. *Nutrition Revs.* **2,** 309 (1944).
188. *Nutrition Rev.,* **4,** 58 (1946).
189. Oleson, J. J., Woolley, D. W., and Elvehjem, C. A. 1939. *Proc. Soc. Exptl. Biol. Med.* **42,** 151.
190. Oppel, T. W. 1942. *J. Clin. Investigation* **21,** 630.
191. Oser, B. L., and Melnick, D. 1945. *J. Nutrition* **30,** 385.
192. Oser, B. L., Melnick, D., and Hochberg, M. 1945. *Ind. Eng. Chem., Anal. Ed.* **17,** 405.
193. Oser, B. L., Melnick, D., and Pader, M. 1943a. *Ind. Eng. Chem., Anal. Ed.* **15,** 717.
194. Oser, B. L., Melnick, D., and Pader, M. 1943b. *Ind. Eng. Chem., Anal. Ed.* **15,** 724.
195. Oser, B. L., and Oser, M. 1946. Unpublished studies.
196. Parsons, H. T. 1946. Gibson Island Conference, Am. Assoc. Advancement Sci.
197. Parsons, H. T., and Collord, J. 1942. *J. Am. Dietet. Assoc.* **18,** 805.
198. Parsons, H. T., Collord, J., Gardner, J., Strong, F. M., and Peterson, W. H. 1942. *Federation Proc.* **1,** 129.
199. Parsons, H. T., Foeste, A., and Gilberg, H. 1945a. *J. Nutrition* **29,** 383.
200. Parsons, H. T., Williamson, A., and Johnson, M. L. 1945b. *J. Nutrition* **29,** 373.
201. Pavcek, P. L., and Shull, G. M. 1942. *J. Biol. Chem.* **146,** 351.
202. Pepkowitz, L. P. 1943. *J. Biol. Chem.* **151,** 405.
203. Polgár, A., and Zechmeister, L. 1942. *J. Am. Chem. Soc.* **64,** 1856.
204. Polgár, A., and Zechmeister, L. 1943. *J. Am. Chem. Soc.* **65,** 1522.
205. Polgár, A., and Zechmeister, L. 1944. *J. Am. Chem. Soc.* **66,** 137.
206. Polgár, A., and Zechmeister, L. 1945. *J. Am. Chem. Soc.* **67,** 108.
207. Poth, E. J., Knotts, F. L., Lee, J. T., and Inui, F. 1941. *Proc. Soc. Exptl. Biol. Med.* **48,** 129.
208. Quackenbush, F. W., Kummerow, F. A., Platz, B. R., and Steenbock, H. 1942. *J. Nutrition* **24,** 225.
209. Quaife, M. L. 1944. *J. Am. Chem. Soc.* **66,** 308.
210. Reder, R. 1946. *Science* **103,** 201.
211. Reinhold, J. G., Nicholson, J. T. L., and Elsom, K. O. 1944. *J. Nutrition* **28,** 51.
212. Robbins, W. J. 1938. *Proc. Natl. Acad. Sci. U. S.* **24,** 53.
213. Robbins, W. J., and Kavanagh, F. 1938a. *Proc. Natl. Acad. Sci. U. S.* **24,** 141.
214. Robbins, W. J., and Kavanagh, F. 1938b. *Proc. Natl. Acad. Sci. U. S.* **24,** 145.
215. Robinson, W. B., and Stotz, E. 1945. *J. Biol. Chem.* **160,** 217.
216. Robinson, W. D., Melnick, D., and Field, H. Jr. 1940. *J. Clin. Investigation* **19,** 399.
217. Roe, J., and Kuether, C. A. 1943. *J. Biol. Chem.* **147,** 399.
218. Rosen, F., Huff, J. W., and Perlzweig, W. A. 1946. *J. Biol. Chem.* **163,** 343.
219. Rowntree, J. I. 1931. *J. Nutrition* **3,** 345.
220. Rubin, S. H. 1946. Personal communication.
221. Rubin, S. H., Scheiner, J., Drekter, L., and Hirschberg, E. 1946. *Abstracts,*

Div. Biol. Chem., Am. Chem. Soc. 33B.
222. Rubin, S. H., Scheiner, J., and Hirschberg, E. 1947. *J. Biol. Chem.* **167,** 599.
223. Russell, R. A., and Nasset, E. S. 1941. *J. Nutrition* **22,** 287.
224. Russell, W. C., Taylor, H. W., Walker, H. A., and Polskin, L. J. 1942. *J. Nutrition* **24,** 199.
225. Salmon, W. D. 1941. *J. Biol. Chem.* **140,** cix.
226. Sarett, H. P., and Cheldelin, V. H. 1945. *J. Biol. Chem.* **159,** 311.
227. Sarett, H. P., Huff, J. W., and Perlzweig, W. A. 1942. *J. Nutrition* **23,** 23.
228. Sarett, H. P., Klein, J. R., and Perlzweig, W. A. 1942. *J. Nutrition* **24,** 295.
229. Sarett, H. P., and Perlzweig, W. A. 1943. *J. Nutrition* **25,** 173.
230. Sargent, F., Robinson, P., and Johnson, R. E. 1944. *J. Biol. Chem.* **153,** 285.
231. Sarma, P. S., Snell, E. E., and Elvehjem, C. A. 1946. *J. Biol. Chem.* **165,** 55.
232. Sarma, P. S., Snell, E. E., and Elvehjem, C. A. 1947. *J. Nutrition* **33,** 121.
233. Schmidt, W., and Schmidt, C. L. A. 1930. *Univ. Calif. Pub. Physiol.* **7,** 211.
234. Schneider, H., Steenbock, H., and Platz, B. R. 1940. *J. Biol. Chem.* **132,** 539.
235. Schultz, A. S., Atkin, L., and Frey, C. N. 1942. *Ind. Eng. Chem., Anal. Ed.* **14,** 35.
236. Schultze, M. O., Stotz, E., and King, C. G. 1938. *J. Biol. Chem.* **122,** 395.
237. Scott, M. L., Hill, F. W., Norris, L. C., and Heuser, G. F. 1946. *J. Biol. Chem.* **165,** 65.
238. Scott, M. L., Randall, F. E., and Hessel, F. H. 1941. *J. Biol. Chem.* **141,** 325.
239. Sealock, R. R. 1945. Gibson Island Conference, Am. Assoc. Advancement Sci.
240. Sealock, R. R., and Goodland, R. L. 1944. *J. Am. Chem. Soc.* **66,** 507.
241. Sherman, W. C. 1941. *J. Nutrition* **22,** 153.
242. Shields, J. B., Johnson, B. C., Hamilton, T. S., and Mitchell, H. H. 1945. *J. Biol. Chem.* **161,** 351.
243. Shohl, A. T. 1936. *J. Nutrition* **11,** 275.
244. Shourie, K. L., and Swaminathan, M. 1940. *Indian J. Med. Research* **27,** 679.
245. Silber, R. H. 1945. *Arch. Biochem.* **7,** 329.
246. Singal, S. A., Briggs, A. P., Sydenstricker, V. P., and Littlejohn, J. M. 1946. *J. Biol. Chem.* **166,** 573.
247. Singher, H. O., Kensler, C. J., Levy, H., Poore, E., Rhoads, C. P., and Unna, K. 1944. *J. Biol. Chem.* **154,** 69.
248. Skeggs, H. R., and Wright, L. D. 1944. *J. Biol. Chem.* **156,** 21.
249. Smith, M. C., and Caldwell, E. 1945. *Science* **101,** 308.
250. Snell, E. E., and Guirard, B. M. 1943. *Proc. Natl. Acad. Sci. U. S.* **29,** 66.
251. Snell, E. E., and Rannefeld, A. N. 1945. *J. Biol. Chem.* **157,** 475.
252. Sobel, A. E., Gottfried, S. P., and Kramer, B. 1946. *Abstracts, Div. Biol. Chem., Am., Chem. Soc.* 28B.
253. Sobel, A. E., Mayer, A. M., and Kramer, B. 1945. *Ind. Eng. Chem., Anal. Ed.* **17,** 160.
254. Sobel, A. E., and Werbin, H. 1945. *J. Biol. Chem.* **159,** 681.
255. Sobotka, H., Kann, S., and Loewenstein, E. 1943. *J. Am. Chem. Soc.* **65,** 1959.
256. Somogyi, J. C. 1945. *Helv. Physiol. Pharmacol. Acta, Compt. rend.* **3,** 15.
257. Spector, H., Hamilton, T. S., and Mitchell, H. H. 1945. *J. Biol. Chem.* **161,** 145.
258. Spector, H., and Mitchell, H. H. 1946. *J. Biol. Chem.* **165,** 37.
259. Spies, T. D., Stanbery, S. R., Williams, R. J., Jukes, T. H., and Babcock, S. H. 1940. *J. Am. Med. Assoc.* **115,** 523.
260. Stewart, A. P., Jr., and Sharp, P. F. 1945. *Ind. Eng. Chem., Anal. Ed.* **17,** 373.
261. Stirn, F. E., Arnold, A., and Elvehjem, C. A. 1939. *J. Nutrition* **17,** 485.

262. Stokes, J. L. 1944. *J. Bact.* **48,** 201.
263. Stokes, J. L., and Martin, B. B. 1943. *J. Biol. Chem.* **147,** 483.
264. Strong, F. M., and Carpenter, L. E. 1942. *Ind. Eng. Chem., Anal. Ed.* **14,** 909.
265. Sure, B. 1944. *J. Nutrition* **27,** 447.
266. Sure, B., and Ford, Z. W. 1942. *J. Biol. Chem.* **146,** 241.
267. Sydenstricker, V. P., Singal, S. A., Briggs, A. P., DeVaughn, N. M., and Isbell, H. 1942. *J. Am. Med. Assoc.* **118,** 1199.
268. Tennet, D. M., and Silber, R. H. 1943. *J. Biol. Chem.* **148,** 359.
269. Thompson, H. O., and Lee, C. O. 1945a. *J. Am. Pharm. Assoc.* **34,** 135.
270. Thompson, H. O., and Lee, C. O. 1945b. *J. Am. Pharm. Assoc.* **34,** 138.
271. Todhunter, E. N., and Fatzer, A. S. 1940. *J. Nutrition* **19,** 121.
272. Totter, J. R., Mims, V., and Day, P. L. 1944. *Science* **100,** 223.
273. U. S. Dept. Agr. 1945. *Misc. Publ. No.* **572,** 30 pp.
274. U. S. Pharmacopoeia, Twelfth Revision. Mack Printing Co., Easton, Pa. (1942).
275. U. S. Pharmacopoeia XII, 1st supplement (1943)
276. Walker, R., and Nelson, E. M. 1933. *Am. J. Physiol.* **103,** 25.
277. Welch, A. D., Heinle, R. W., Nelson, E. M., and Nelson, H. V. 1946. *J. Biol. Chem.* **164,** 787.
278. Welch, A. D., and Wright, L. D. 1943. *J. Nutrition* **25,** 555.
279. Weswig, P. H., Freed, A. M., and Haag, J. R. 1946. *J. Biol. Chem.* **165,** 737.
280. Whipple, D. V., and Church, C. F. 1935. *J. Biol. Chem.* **109,** xcviii.
281. Williams, R. R. 1939. The Vitamins. Am. Med. Assoc. 141.
282. Williams, V. R., and Vieger, E. A. 1945. *Ind. Eng. Chem., Anal. Ed.* **17,** 127.
283. Williamson, A., and Parsons, H. T. 1945. *J. Nutrition* **29,** 51.
284. Wilson, R. H., Ambrose, A. M., De Eds, F., Dutton, H. J., and Bailey, G. F. 1946. *Arch. Biochem.* **10,** 131.
285. Wintrobe, M. M., Stein, H. J., Follis, R. H., and Humphreys, S. 1945. *J. Nutrition* **30,** 395.
286. Woolley, D. W. 1939. *J. Biol. Chem.* **130,** 417.
287. Woolley, D. W. 1942. *J. Exptl. Med.* **75,** 277.
288. Woolley, D. W. 1946a. *Advances in Enzymol.* **6,** 129.
289. Woolley, D. W. 1946b. *J. Biol. Chem.* **163,** 773.
290. Woolley, D. W., and Longsworth, L. G. 1942. *J. Biol. Chem.* **142,** 285.
291. Wright, L. D., and Skeggs, H. R. 1944. *Proc. Soc. Exptl. Biol. Med.* **56,** 95.
292. Wright, L. D., Skeggs, H. R., and Welch, A. D. 1945. *Arch. Biochem.* **6,** 15.
293. Wright, L. D., and Welch, A. D. 1944. *J. Nutrition* **27,** 55.
294. Young, R. M., and James, L. H. 1942. *J. Bact.* **44,** 75.
295. Young, R. M., and Rettger, L. F. 1943. *J. Bact.* **46,** 351.
296. Zechmeister, L., Le Rosen, A. L., Schroeder, W. A., Polgár, A., and Pauling, L. 1943. *J. Am. Chem. Soc.* **65,** 1940.

Thiamine and Peripheral Neurophysiology

By ALEXANDER VON MURALT—(Bern)

CONTENTS

Page
I. Introduction 93
II. Liberation of Thiamine in Nervous Excitation 93
III. Thiamine Content of Peripheral Nerves 105
IV. Liberation of Thiamine on Vagus Stimulation in the Heart 110
V. Discussion and Summary 114
References 116

I. INTRODUCTION

Strange as it may seem, direct evidence that thiamine plays an important rôle in the chemical mechanism of nervous excitation was not presented until 1938. Indirect evidence of such a correlation, however, goes back to the work of Eijkman (1897) and Grijns (1901) in the Dutch East Indies, at a time when nobody had even thought of "vitamins." They recognized that it was the lack of a certain dietary factor(s) present in rice bran, which produced avian polyneuritis and beri-beri. We would say today that the lack of thiamine produces a fundamental change in the chemical mechanism of each neurone, by which its function is dangerously affected. As soon as the factor is replaced, the chemical equilibrium of the neurone is restored and, with this assumption, one is entitled to call the factor an "antineuritic principle," as Eijkman (1897) has done. We would say today: thiamine is essential for the maintenance of the internal chemical equilibrium of the neurone (nerve cell and peripheral nerve). The store of thiamine in the cell is continuously used up in maintaining this equilibrium, and, as the cell is not able to synthesize this vitamin, a constant requirement from other sources is the result. What is the rôle of thiamine in the chemical equilibrium of the neurone? Why is thiamine permanently used up in maintaining this equilibrium? What is the requirement of a neurone at rest and during activity? These questions seem so simple, and yet it is so hard to give an answer which corresponds to a first approximation of the problem!

This article has been written in an attempt to show the methods and procedures which have been employed since 1938 in an attempt to answer these three questions.

II. LIBERATION OF THIAMINE IN NERVOUS EXCITATION

In 1938 Minz (1938a,b) published a few experiments with excised ox nerves, which were placed in a physiological solution after the surfaces had been cut. Electrical stimulation was applied to the nerves while their ends were dipped in the bath.. The solution was tested for thiamine content by the *Flagellatae* test. *Polytomella caeca,* which was used in one group of experiments can only grow if thiamine, or the two fractions of the thiamine molecule, pyrimidine and thiazole, are present in the medium, *i.e.*, this organism can synthesize thiamine from its two fractions. *Glaucoma piriformis,* which was used in another experiment, requires the whole molecule for growth and cannot use the fractions alone. The solution was collected and tested after stimulating the nerve-trunks for 10 minutes (40 stimuli/sec.), a second portion of the solution, corresponding to an identical period of immersion of the nerves without stimulation, being similarly treated. The results are shown in Table I.

TABLE I

Specimen	Number of organelles grown in 1 mm.^{3}						
	S	U	S	U	S	U	S_{30}
Polytomella	600	75	800	100	200	50	30
Glaucoma	60	10					

The growth effect of the solution, S, from stimulated nerves corresponds to about 1/100 γ of thiamine. The figures show that about 4–8 times more thiamine diffuses from the cut surface while the nerve is stimulated, than when at rest. In one experiment stimulation was tried after 30 minutes, (S_{30} in Table I), but without any further liberation of thiamine. Either the cut surface had lost its permeability or no thiamine had been formed due to inexcitability of the nerve. These few, but important, observations were of the greatest interest to the group in Berne, who had been working since 1936 on acetylcholine liberation in excited peripheral nerves.

Liberation of Acetylcholine in Nervous Excitation. Nachmansohn (1945) has reviewed the rôle of acetylcholine in the mechanism of nerve activity. In 1936 it was a well established fact that cholinergic nerves (motor nerves, preganglionic sympathetic, parasympathetic, and postganglionic parasympathetic nerves) liberate acetylcholine at their terminals on stimulation (Loewi, 1937; Dale, 1935). At that time it was also evident, but not generally known and accepted, that the cut surfaces of nerves liberated acetylcholine into bathing fluids on stimulation (Calabro, 1933; Bergami,.1936a,b; Binet and Minz, 1934; Rossine, 1936a, 1936b). From this observation the conclusion could be drawn that acetylcholine is formed along the entire length of the nerve during excitation. However, Gaddum *et al.* (1937) published a paper in which they came to the conclusion: "The release of the active substance is surprising, but does not reveal a physiological mechanism. The main object of this communication is to draw attention to this local phenomenon at the electrodes. The methods used by the workers mentioned above (Calabro, Bergami, Binet and Minz) would not discriminate between effects due to the passage of impulses, and effects due to local changes at the electrodes." It was, therefore, necessary to prove that acetylcholine is formed in excited nerves by using a method which leaves no doubt of its nature. If the excitation wave is associated with, or followed by, a chemical wave, it must be possible to determine the amount of acetylcholine by freezing excited nerves in liquid air and thereby fixing the state of excitation. The first results obtained by a special technique were published 3 months after Gaddum's paper by Muralt (1937). By this technique the chemical waves were not only frozen, but also accumulated in the nerve. The acetylcholine content of nerves frozen by this technique were compared with the content of their controls, taken from the same animal, but frozen at rest. Special precautions had to be taken because liquid air itself acts as a stimulus. The "resting" nerves were made unexcitable by the slow action of cold, by cocaine or by ether vapors. These experiments showed (Muralt, 1937; Muralt *et al.*, 1938; Muralt, 1939a,b,c; 1942; 1946) that excited frog nerves contain 0.24 $\gamma \pm 0.01$ of acetylcholine, whereas unexcited nerves had only 0.15 $\gamma \pm 0.01$ (average of 50 independent determinations with calculated errors). The difference of less than 0.1 γ seems to be small and it is questionable whether such a difference is real. The mean error $\Sigma(D)$ for a difference D between two mean values M_1 and M_2 was calculated

$$\Sigma(D) = \pm \sqrt{\Sigma(M_1)^2 + \Sigma(M_2)^2}$$

and the quotient $D/\Sigma(D)$ determined. A difference must be regarded as insignificant if $D/\Sigma(D)$ is smaller than 2, "probable" if greater than 2 but smaller than 3, and "certain" if greater than 3 (Norberg, 1942). In the case of the acetylcholine determination the quotient $D/\Sigma(D)$ was 7, *i.e.*, the difference is reliable and real. With each excitation wave traveling down a nerve, 0.1 γ of acetylcholine is "liberated" per g. of nerve in a sort of "chemical wave" and almost as quickly inactivated during the refractory period following each excitation. The determinations of acetylcholine were made with: (*a*) the leech preparation and (*b*) the response of the frog-heart. To the Bernese group it was obvious in 1938 that another unknown factor was interfering with the determinations of acetylcholine, especially in nerve extracts. Extracts made from excited nerves showed a very considerable difference between method (*a*) and (*b*), the leech method yielding higher values than did the frog heart method. It was also surprising that these differences were greater in summer than in winter months. As soon as Minz published his results it became clear that thiamine might be interfering in the determination of acetylcholine. Thiamine has a sensitizing action for acetylcholine on eserinized leech muscle (Binet and Minz, 1934; Brücke and Sarkander, 1940; Muralt, 1942), so that an excessive amount of acetylcholine is found in extracts containing both. On the frog heart, thiamine has an inhibiting action for acetylcholine (Agid and Balkanyi, 1938; Kaiser, 1939; Moniz de Bettencourt and Paes, 1936a,b), *i.e.*, the negative chronotropic and inotropic effects of acetylcholine are decreased if thiamine is present at the same time and too low an amount of acetylcholine is determined. It became evident that thiamine is liberated at the same time as acetylcholine and that the amount liberated had to be determined. The figures given above for acetylcholine liberation have been corrected for the "thiamine effect."

Determination of Thiamine Liberation in Excited Nerves. Beginning in 1939 the Bernese group applied the technique of freezing nerves in the excited state to the determination of thiamine liberated during or shortly after excitation. Since the amounts to be determined are of the order of 0.01 γ, very refined methods had to be used. The evidence so far obtained is based on: (*a*) direct determination using specific thiamine methods: bradycardia test (Liechti *et al.*, 1943), *Phycomyces* test (Muralt and Zemp, 1943), yeast fermentation test (Wyss and Wyss, 1945a,b) and the yeast micro test by the diver method (Went, 1946); (*b*) direct determination with nonspecific thiamine methods: thiochrome method using a highly sensitive electric fluorometer (Wyss, 1943; 1944), sensitizing effect on the leech muscle (Muralt, 1942), and polarographic determination (Muralt, 1941; 1942; Weidmann, 1945; 1946); and (*c*) indirect evidence gathered from experiments with ultraviolet light, making use of the specific absorption of thiamine, of its transformation into thiochrome, and of the photochemical decomposition of thiamine. A description of the freezing method and the methods used for thiamine determination has been given in full detail elsewhere (Muralt, 1946).

The results obtained with these methods are interesting and in one point unexpected. Fig. 1 is an example of experiments with the bradycardia test. The extracts of 50 frozen nerves (50 excited and 50 unexcited) were fed to various groups of rats in a state of avitaminosis. The average increase of pulse rate after having fed the extracts on day 0 is plotted as a function of time, together with controls of standard thiamine preparations and a blank. It is obvious that the extracts from stimulated nerves contain a measurable amount of thiamine, whereas the extracts of unstimulated nerves contain only a subthreshold amount of thiamine, if any is present.

At this point of the discussion a very important point in procedure must be mentioned to clarify the meaning of the words "extract" and "liberated." The frozen nerves are ground in a mortar until a very fine powder is obtained and then brought to the melting point. At this moment, to 1 cc. of Ringer solution, 10 mg. of nerve-powder is added and left in contact with the powder for only 10 minutes. The extract is then filtered off or separated from the powder by centrifuging in such a way that the contact between powder and solution is never longer than 15 minutes. The amount of thiamine found in the solution is, therefore, a measure of the soluble fraction rather than of the actual concentration in the nerve powder. If we say "thiamine is liberated" it really means that *thiamine in the powder of excited nerves is in such a state, that more can be extracted by Ringer solution in 10 minutes than can be obtained from a corresponding sample of unexcited nerves.*

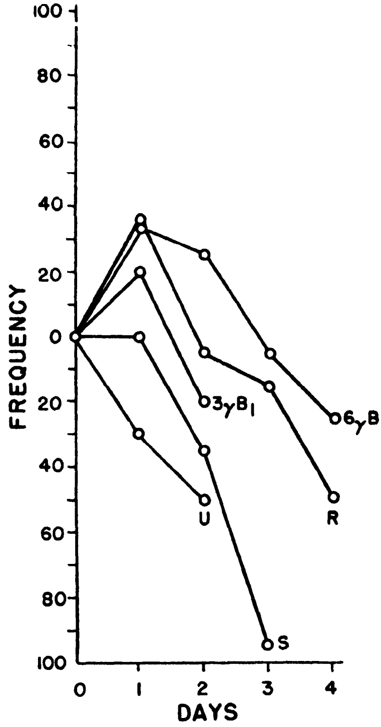


FIG. 1.—Thiamine in excited and unexcited nerves, determined by the bradycardia test. Ordinate: average pulse rate increase and decrease reduced to starting pulse rate. Abscissa: time in days. Ordinate: pulse rate. R = extract of excited nerves; U-extract of unexcited nerves; S = blank; B_1 = thiamine tests.[a]

Fig. 2 shows a corresponding experiment with the *Phycomyces* test. The growth curves of *Phycomyces* are plotted for varying standard thiamine concentrations and also for extracts of excited and unexcited nerves. The same difference was obtained and the determinations gave an average "liberation" of thiamine of 1–2 γ/g. of excited nerve. This is surprising, considering that the acetylcholine "liberation" only yielded 0.1 γ/g. Thiamine is "liberated" in 10–20-fold greater amounts than acetylcholine. The same

---

[a] Note: Figs. 1, 2, 6 and 7 are taken from Muralt's "Signalübermittlung im Nerven," pp. 295, 297, 106 and 107, respectively.

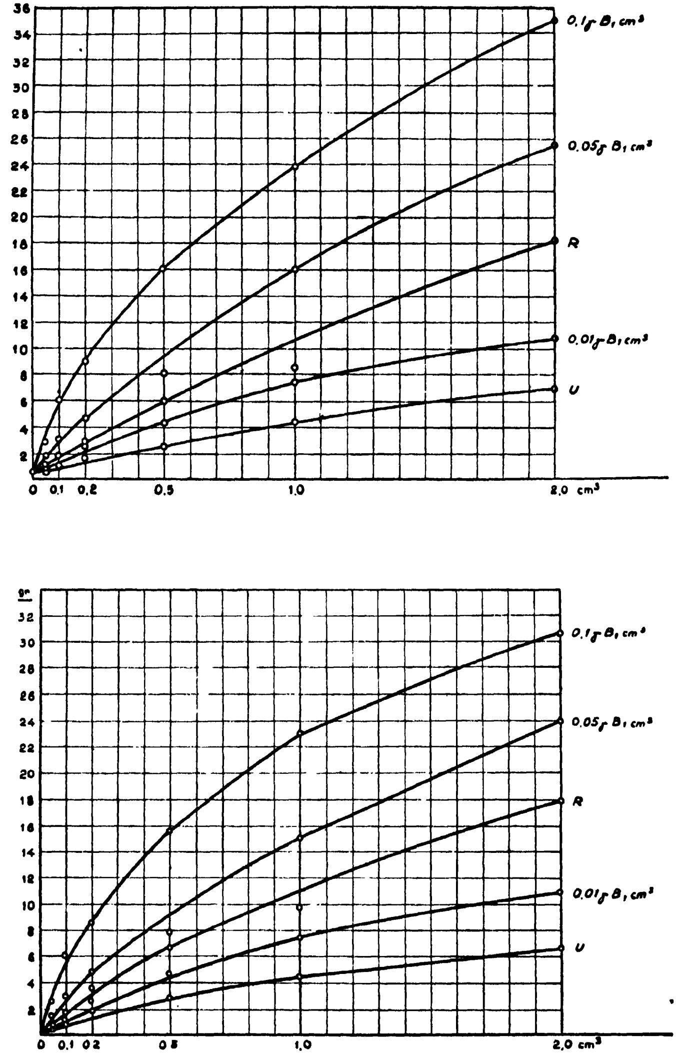


FIG. 2.—Thiamine in excited and unexcited nerves, determined by the *Phycomyces* test. Ordinate: weight of mycelia in g.; abscissa: amount of tested solution; R = extract of excited nerves, U = extract of unexcited nerves. The curves combine the values obtained with the same concentration but in different amounts. To show the reproducibility, two sets of curves from independent experiments are presented (Muralt, 1946).

result was obtained in the experiments with the 3 unspecific methods mentioned previously and it seems to be well established that a considerable amount of thiamine is "liberated" during the process of excitation.

The first surprise was the observation of Wyss and Wyss (1945b) that, after poisoning a nerve with monoiodoacetic acid, more thiamine is obtained in the extract of resting nerves than in the extract of excited nerves. In the poisoned nerve thiamine is "liberated" in the resting metabolism and "fixed" in the process of excitation, *i.e.*, under conditions in which the sequence of chemical reactions has been altered by the poisoning, the poisoned nerve shows a behavior opposite to that of the normal nerve.

The second surprise was the fact that the yeast fermentation method used, either according to Atkin, Schultz and Frey (1939) or in the highly refined form of the micro diver method (Holter and Linderström-Lang, 1943; Went, 1946), gave no difference between "excited" and "unexcited" extracts and much lower values for the thiamine content of the solutions. Addition of thiamine to nerve extracts, however, always yielded correct values, so that an "inhibitory" effect can be excluded. The fact that the yeast method does not show any signs of thiamine liberation in excited nerves is a very important finding and throws some doubt on the previous observations by 6 independent, and more or less specific, methods. No explanation for this discrepancy can be offered today. Only experimental work can show whether yeast has special properties with regard to thiamine derived from nerves, or whether the extracts contain other factors which cause the abnormal reaction.

Keeping this in mind, we should now consider the indirect evidence for the important role of thiamine in nervous conduction. Thiamine has a very characteristic ultraviolet absorption band near 260 mμ. At the same time thiamine is photochemically destroyed by the action of shortwave ultraviolet, but not by long-wave ultraviolet (Ruehle, 1939; Uber and Verbrugge, 1940; Stämpfli, 1942.) Under the influence of alkali and potassium ferricyanide, thiamine is transformed into thiochrome (Kuhn *et al.*, 1935; Jansen, 1936) and this substance shows a very strong fluorescence. These facts were the starting points for various investigations.

Caspersson (1940) was the first to apply methods of ultraviolet absorption to cytological problems. An apparatus for measuring the ultraviolet absorption of living, single nerve fibers was built according to his design, but with certain improvements (Muralt, 1946; Lüthy, 1946a). Fig. 3 shows the absorption curve of a living nerve fiber in ultraviolet light. There is a first maximum between 280–285 mμ which is due to the protein of the nerve, especially the tyrosine in the protein. The second maximum at 265 mμ is due to nuclein components and, in part, to the thiamine content of nerve. It can be shown that treating the nerve with potassium ferricyanide and thereby transforming thiamine into thiochrome reduces this band considerably. (Thiochrome has no specific band at 265 mμ.) A very interesting fact was brought out by the experiments of Lüthy (1946b) who found in the living nerve a very marked dichroism in the ultraviolet region! Fig. 4 shows the absorption curve of medullated nerve-fibers (frog) under polarized light. If the electric vector is parallel to the fiber, the absorption at 265 mμ is greater than if it is perpendicular. At 280 mμ exactly the reverse effect is observed. If we say that by and large the 280 mμ absorption is due to protein structures and at 265 mμ to lipoid structures, it would follow that the absorbing elements in these structures are oriented at right angles. And that is exactly what Schmitt's (1939) studies of double refraction and X-ray patterns of the myelin sheath of medullated nerves have shown!

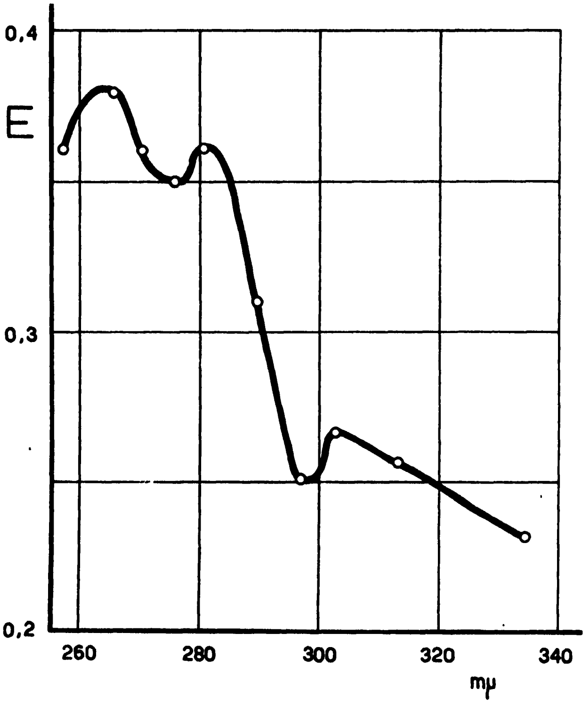


FIG. 3.—Ultraviolet absorption of single nerve fibers of the frog. Ordinate: extinction coefficient (E); abscissa: wave length of light (mμ).

If the interpretation is correct, then a non-medullated nerve should produce a different picture. This is shown in Fig. 5 which was obtained from the olfactorius of the pike. This nerve consists of nerve fibers (axones) immersed in a myelin-like environment, *without* protein-myelin structure. The axones have a definite protein structure, but parallel to the fiber. The protein leaflets which alternate with lipoid layers in the sheath of medullated nerves are not developed in these nerves. The ultraviolet absorption of the lipoid component is, therefore, unchanged with regard to the character of dichroism, the protein component however is reversed, just as must be expected, because the dominating effect of the protein leaflets is missing and the axone structure shows its dichroism exposed. With electric excitation very specific effects can be observed in these living nerve fibers with regard to their dichroism.

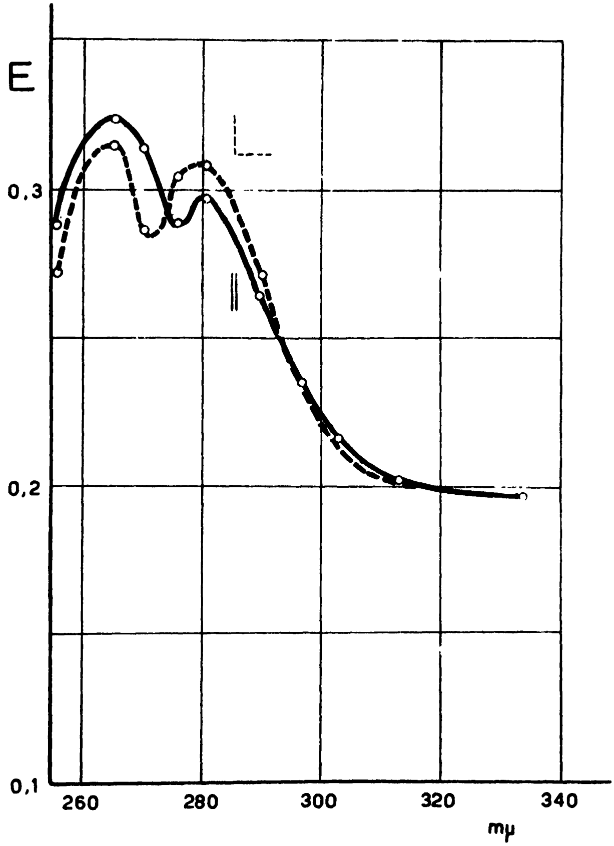


FIG. 4.—Ultraviolet absorption of single nerve fibers of the frog in polarized light. Ordinate: extinction coefficient (E); abscissa: wave length of light (mμ). The orientation of the electrical vector with respect to the fiber axis is indicated by signs.

These experiments are in progress but it is still too early to say much about them. They seem to show in a very conclusive way that, with and after excitation, a molecular regrouping takes place in the protein and in the lipoid system, together with the thiamine component. The "liberation" of thiamine is not only a question of a change of solubility but also in its fundamental nature in the nerve. From this point of view the experiments described previously take on increasing importance.

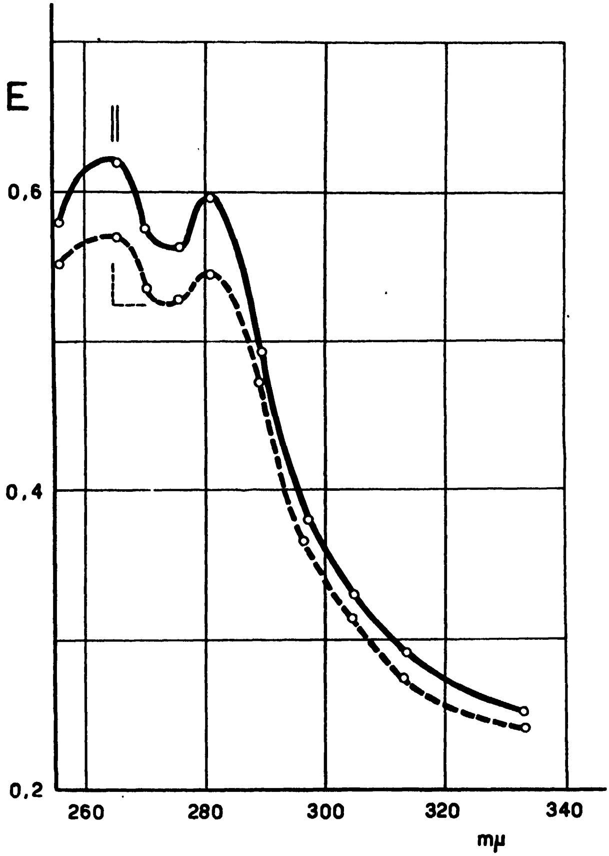


FIG. 5.—Ultraviolet absorption of the olfactory nerve of the pike in polarized light. Ordinate: extinction coefficient (E); abscissa; wave length of light (mμ).

The photochemical sensitivity of thiamine led to another group of experiments of considerable theoretical interest (Hutton-Rudolph, 1943; 1944). Single living nerve fibers were irradiated with ultraviolet light. At the same time their excitability was measured by determining the threshold and chronaxy of these nerves. Fig. 6 shows one experiment in which the light was filtered by only a quartz cuvette containing distilled water. The very constant values for the rheobase show that the single nerve fiber was in an excellent state up to the time of irradiation. Two phases of the irradiation effect were always observed. We called them phase of increased excitability and phase of destruction. The fact that only ultraviolet light of less than 300 mμ produces these specific effects, showed at once that they are due to a specific absorption in the nerve. This can be shown by a very interesting effect. If a cuvette containing a substance with a specific absorption is used as a light filter, the best protective action is produced by that substance which has the same absorption as the photochemically sensitive substance in the nerve, *i.e.*, by the substance itself. We found, among all substances investigated, that thiamine has an absolutely ideal protective action for, as long as the ultraviolet is passed through a 1% thiamine filter, all those wave-lengths which have photochemical action on the nerve fiber (Fig. 7) are absorbed before reaching the fiber and cannot excite its action. Those wave-lengths which are not absorbed by a 1% thiamine filter 0.5 cm. in thickness pass without any decrease in intensity, but have no photochem-

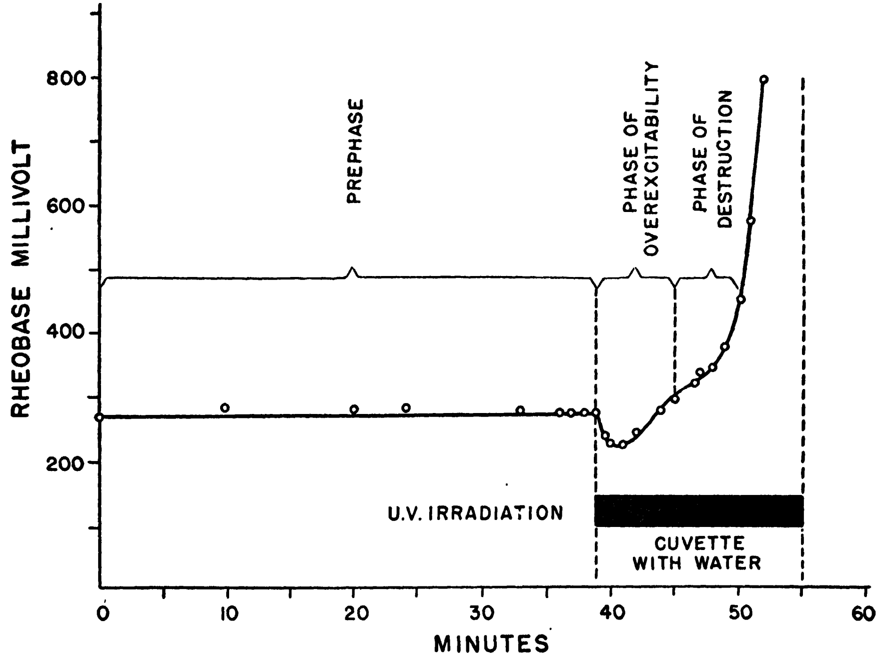


FIG. 6.—Photochemical action of short wave ultraviolet on the excitability of a single nerve fiber. Ordinate: rheobase in millivolts; abscissa; time in minutes. The irradiation begins after 40 minutes; typical results of 32 experiments (Muralt, 1946).

ical action on the nerve fiber. As soon as the filter is replaced by a filter with distilled water the photochemical effect begins. The conclusions from a great number of such experiments were: the first phase of the photochemical reaction (overexcitability) is due to the destruction of a substance with an absorption spectrum similar to, or identical with, that of thiamine. This substance is localized in the sheath of medullated nerves. The phase of overexcitability corresponds to the effect produced in nerve *in vivo* by the initial phase of thiamine avitaminosis. The phase of destruction corresponds to denaturation of the nerve proteins. Irradiation of the node of Ranvier alone, where there is only protein and no

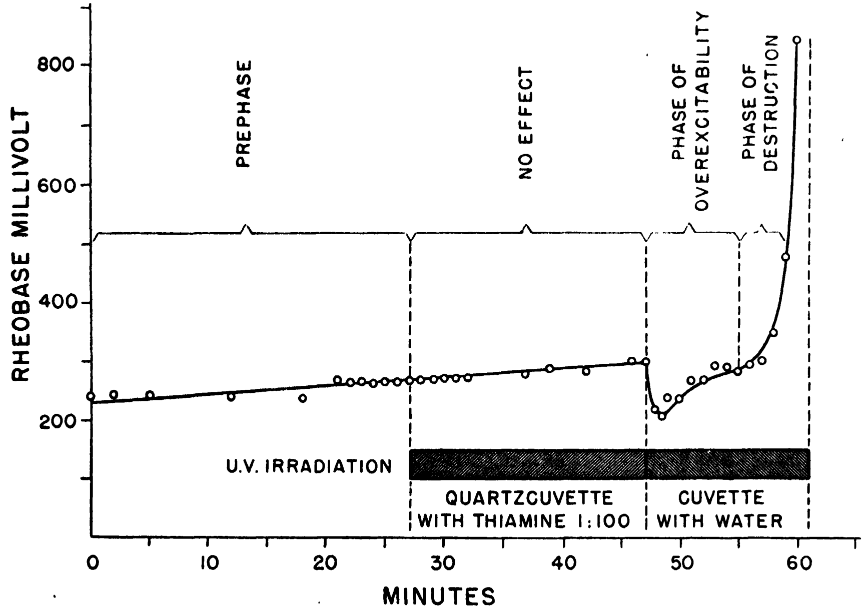


FIG. 7.—Protective action of a thiamine filter against the photochemical action of short wave ultraviolet on the single nerve fiber. Ordinate: rheobase in millivolts; abscissa: time in minutes. The light passed through a thiamine solution before falling on the nerve fiber from 27th–47th minute (Muralt, 1946).

lipoid or thiamine produces only the second phase. One should not draw too far-reaching conclusions, but it is obvious to those who have been working with single nerve fibers and ultraviolet that there is a close relationship between thiamine destruction and change of excitability of the nerve.

If histological preparations of nerves are made with alkali and potassium ferricyanide and neutralized, a definite blue fluorescence is observed (Muralt, 1943). To a great degree this is due to the transformation of nerve thiamine into thiochrome. Fig. 8 shows a preparation of a single fiber in the fluorescent microscope. The node of Ranvier has no fluorescence at all and the fluorescent substance is localized in the medullary sheath. This can be interpreted as showing that thiamine is stored in the nerve sheath and not in the axone and corresponds to the finding of a specific ultraviolet dichroism at 265 mμ. Nachmansohn and Steinbach (1942) studied the giant axone of the squid and found that cocarboxylase is concentrated on the surface of the axone. If the nerves are in electrotonus at the moment of fixation, an accumulation of thiamine is observed at the site of the anode, which is an indication that thiamine is liberated in relation to anabolic, rather than to catabolic, processes.

All these findings support the view that thiamine plays an important role in nerve metabolism and that *during the state of excitation or shortly afterwards* a certain "liberation" of thiamine takes place which disappears almost as quickly as it is produced. Such is the experimental evidence today. It must be stressed that the findings are fragmentary and their interpretation might be influenced by further experimental work.

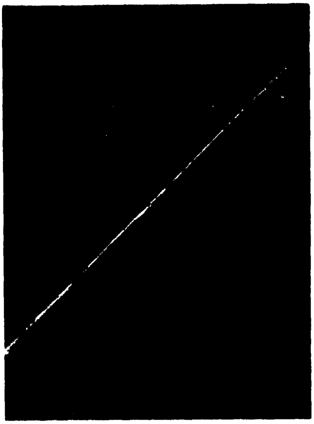


FIG. 8.—Fluorescence of a single nerve fiber in which, by Muralt's technique, the thiamine was previously oxidized to thiochrome. Photomicrograph taken in fluorescent light, 5 minutes exposure.

III. THIAMINE CONTENT OF PERIPHERAL NERVES

Thus far no distinction has been made between thiamine and the various forms in which thiamine can exist in the cell, such as thiamine diphosphate (cocarboxylase), thiamine monophosphate, thiamine sulfide and disulfide, *etc.* There is also at least one form, and possibly more, of bound thiamine. The content of nerves with regard to free and bound thiamine is of special interest. As far as could be gathered these findings have been summarized in Table II. The body is unable to store thiamine to any extent, so that the figures given correspond to the normal and, at the same time, nearly the maximum amount in each nerve. "Free thiamine" is the moiety which can be extracted in aqueous solutions without special methods, "bound thiamine" is the amount (total *minus* free thiamine) which can be determined only after acid extraction, or by the *Phycomyces* method, in which case it is assumed that *Phycomyces* is able to make use of the total amount of vitamin present. The data are

TABLE IIa

Thiamine, Free and Bound, in Nerves and Brain

Animal	Tissue	Thiamine (free) γ/g.	Thiamine (bound) γ/g.	Method	Author
Frog........	Sciatic	1.2	2.5	Thiochrome (manometric)	Wyss (1944)
	Sciatic	....	0.6 (unstimulated nerves) to 2.7 (stimulated nerves)	*Phycomyces*	Muralt and Zemp (1943)
Rat.........	Brain	0.3	6.0	Thiochrome	Westenbrink and Goudsmit (1938)
	Brain	0.8–1.0	2.5–3.5	Thiochrome	Ritsert (1939)
	Brain	0.3	2.3	Thiochrome (modified)	Wiedenbauer (1939)
	Brain	1.9	3.5	Thiochrome	de Caro and Butturni (1940)
	Brain	0.8	3.1	Thiochrome and *Phycomyces*	Muralt jun. (1946)
	Sciatic	0.7	1.6	*id.*	*id.*
	Cerebellum	0.2	3.4	*id.*	*id.*
	Medulla oblongata	0.5	3.6	*id.*	*id.*
	Spinal cord	1.0	....	Thiochrome	Ritsert (1938)
Pig..........	Obturatorius	0.26	0.39	Thiochrome	Sanz (1943)
Dove........	Brain	0.1	2.5	Thiochrome	de Caro and Butturini (1940)
Guinea pig...	Brain	0.2	3.2	Thiochrome (modified)	Wiedenbauer (1939)
	Sciatic	0.1	1.0	Thiochrome (manometric)	Muralt and Wyss (1944)
Dog.........	Sciatic	0.6	1.1	Thiochrome and *Phycomyces*	Muralt jun. (1946)
	Vagus	0.4	0.9	*id.*	*id.*
	Phrenic	0.4	1.9	*id.*	*id.*
	Spinal cord	0.5	0.5	*id.*	*id.*
	Medulla oblongata	0.7	1.7	*id.*	*id.*

TABLE IIa.—(*Continued*)

Animals	Tissue	Thiamine (free) γ/g.	Thiamine (bound) γ/g.	Method	Author
	Brain (cortex, occipital lobe)	0.6	2.0	*id.*	*id.*
	Thalamus	0.4	1.4	*id.*	*id.*
	Corpus callosum	0.6	0.8	*id.*	*id.*
	Corpora quadrigemina	0.5	0.8	*id.*	*id.*
	Cerebellum (vermis)	0.7	1.4	*id.*	*id.*
	Splanchnic	0.5	0.7	*id.*	*id.*
	Thoracic sympathetic chain	0.4	0.4	*id.*	*id.*
	Preganglionic sympathetic	$< 1 \times 10^{-7}$	1.0	*id.*	*id.*
Calf.........	Sciatic	0.5	0.7	*id.*	*id.*
	Phrenic	0.4	0.7	*id.*	*id.*
	Splanchnic	0.4	0.9	*id.*	*id.*
	Vagus	0.3	0.5	*id.*	*id.*
	Spinal cord	0.6	0.6	*id.*	*id.*
	Ventral roots	$< 1 \times 10^{-7}$	0.4	*id.*	*id.*
	Dorsal roots (without ganglion)	$< 1 \times 10^{-7}$	0.5	*id.*	*id.*
	Spinal ganglion (without roots)	0.5	0.9	*id.*	*id.*
	Thoracic sympathetic chain (without ganglion)	0.2	0.3	*id.*	*id.*
	Sympathetic ganglion	0.4	0.6	*id.*	*id.*
	Brain (cortex)	0.7	0.8	*id.*	*id.*
	Corpus callosum	0.2	1.3	*id.*	*id.*
	Superior corpora quadrigemina	0.8	1.2	*id.*	*id.*
	Medulla oblongata	0.6	1.4	*id.*	*id.*
	Thalamus	0.9	1.8	*id.*	*id.*

instructive inasfar as they show that in peripheral nerves of warm-blooded animals less than 0.5 γ of free thiamine, and not much more than 1 γ of bound thiamine, is present on the average. In the spinal cord and brain the corresponding figures are at least twice as great. The extent to which seasonal differences influence the values is shown in Table IIb where the same determinations were made on 6 rabbits in winter and on 6 other animals in summer. It is obvious that, as soon as the food provides more vitamin, the storage is on the "bound" side and not on the "free" side of thiamine in brain and nerve.

TABLE IIb

Free and Bound Thiamine in Nerves and Brain of Rabbits

I. Six rabbits of the same age and fed the same diet for three weeks before determination of thiamine. Determinations made in *winter.*

	Free thiamine (Thiochrome) γ/g.	Bound thiamine (*Phycomyces*) γ/g.
Brain (cortex)	0.7	0.9
Mid-brain	0.6	1.4
Medulla oblongata	0.8	1.3
Spinal cord	0.5	0.9
Sciatic	0.4	0.5
Vagus	0.3	0.5
Phrenic	0.2	0.5

II. Six rabbits of different ages and on different diets before determination of thiamine. Determinations made in *summer.*

	Free thiamine (Thiochrome) γ/g.	Bound thiamine (*Phycomyces*) γ/g.
Brain (cortex)	0.6	2.0
Mid-brain	0.4	2.4
Medulla oblongata	0.5	2.9
Spinal cord	0.4	2.1
Sciatic	0.5	0.7
Vagus	0.3	1.0
Phrenic	0.3	0.8

G. v. Muralt, unpublished.

During *degeneration* the nerves loose their thiamine content rather quickly, but more slowly than their acetylcholine, which shows that this valuable chemical component is safeguarded quite well in the nerve. In the blood the level is much lower and corresponds to 0.1 γ/g. and practically all is in the form of cocarboxylase (Westenbrink, 1940), so that there is always a gradient of concentration between blood and tissue. In the degenerating nerve the trophic influence of the nerve cell has been eliminated by cutting or crushing and, therefore, the "protective organization" is lost and the thiamine diffuses out. The thiamine content of the sciatic nerve in the guinea pig has been investigated by Muralt and Wyss (1944) and Fig. 9 shows their results. The operation was always effected on one side of the animal and the thiamine content of the nerve of the operated side is expressed in *per cent* of the content of the unoperated symmetrical nerve. Very striking is the fact, not visible in Fig. 9, that 10 hours after operation no free thiamine was found in the degenerating nerve, whereas in the normal nerve there was always a measurable amount of free thiamine. The rapid loss of thiamine, together with the very rapid loss of acetylcholine, as Brown and Feldberg (1936), McIntosh (1938, 1941), Feldberg (1943) and Muralt and Schulthess (1944) have found, is one of the first signs of the start of degeneration. The relationship between acetylcholine content and thiamine

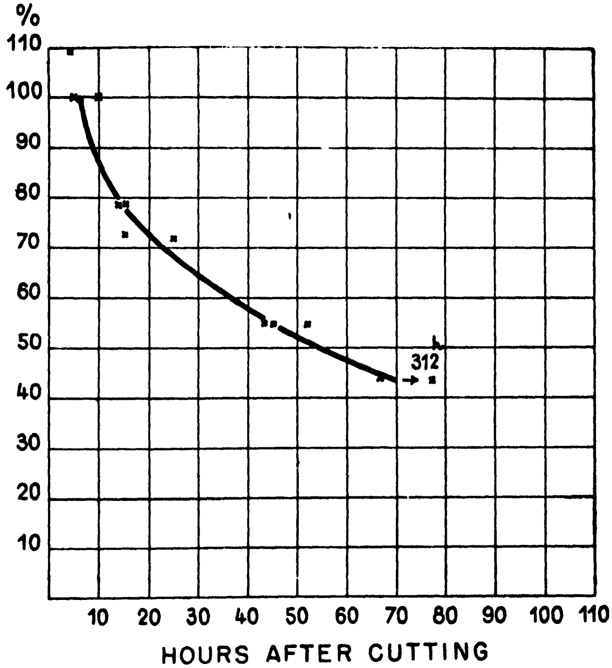


FIG. 9.—Thiamine content of the sciatic nerve (guinea pig) during degeneration. Ordinate: Thiamine content of degenerating nerve in *per cent* of content of the symmetrical nerve; abscissa: hours elapsed from operation to determination.

content is shifted in degeneration. Table III represents the shift in this relation (Muralt, 1946). When the relation reaches the value of 0.2, excitation of the nerve is no longer possible. Thiamine is considered as a storage substance in the nerve, closely related to excitation. If thiamine is administered to the animals during degeneration of the nerve, then it takes more time for the relation acetylcholine: thiamine to fall to the limiting value of 0.2 and the loss of excitability is reduced. The administration of thiamine has been recommended (Muralt, 1943b) in all cases where the risk of nerve degeneration is *impending*, with good results. Degeneration is inevitable if a nerve is cut and the administration of thiamine is not recommended because it does not favorably affect regeneration. The fact that thiamine and acetylcholine are disappearing so quickly when a nerve starts degenerating is regarded as additional proof of the importance of these two substances in the chemical mechanism of excitation. In muscle physiology it is well known that the important substances in the chemistry of contraction (phosphagen, glycogen, *etc.*) are the first ones to be lost with degeneration (Tower, 1939). It is not dangerous to draw the same conclusion with regard to the nerve, although its chemical mechanism is known to a lesser extent.

TABLE III

Relation of Acetylcholine to Thiamine in the Degenerating Nerve

Relation of $\frac{\text{Acetylcholine}}{\text{Thiamine}}$ in *per cent* before cutting nerve	Hours after cutting nerve					
	5 hr.	10 hr.	20 hr.	30 hr.	40 hr.	60 hr.
1.0	0.9	0.8	0.7	0.5	0.4	0.2

IV: Liberation of Thiamine When the Vagus in the Heart Is Stimulated

The first step in the discovery of chemical factors connected with nervous excitation was Loewi's finding (1937) of the liberation of acetylcholine on vagus stimulation of the heart. The second step was the extension of this observation to apply to all endings of cholinergic nerves. The third step was the establishment of the general importance of acetylcholine liberation in the excitation of the whole length of a cholinergic nerve. The role of thiamine was discovered in the reverse order; first the liberation of thiamine along the nerve was discovered and then it was only logical to look for the liberation of thiamine on vagus stimulation of the heart.

It has been mentioned that thiamine inhibits the action of acetylcholine on the heart (Agid and Balkanyi, 1938; Kaiser, 1939; Moniz de Bettencourt, 1936) and thus acts as a brake. Assuming that normally this action counterbalances the action of acetylcholine which is liberated as a result of the natural action of the vagus, then a state of avitaminosis should produce bradycardia. This is exactly what Williams, Mason, Wilder and Smith (1940) observed in man at rest at the beginning of avitaminosis. In beri-beri, however, the tachycardia and arhythmia develop in the more severe cases and it is very probable that these disturbances are the consequence of the abnormal metabolism of the heart *muscle* tissue and have nothing to do with the disturbance of vagus action, which is noticeable only at the beginning of avitaminosis and before the heart tissue is affected (a very comprehensive review has been given by Bicknell and Prescott, 1946). In the rat, the bradycardia is very obvious and is certainly to a large extent related to the disturbance of the normal vagus action by avitaminosis and to a smaller extent to the abnormal metabolism of the heart muscle. These theoretical considerations show that thiamine and vagus action are related.

The experimental evidence for thiamine liberation on vagal stimulation of the frog heart was obtained by Wyss and Muralt (1944), Wyss and Wyss (1945), and Muralt (1945), and offered considerable technical difficulty. Thiochrome and *Phycomyces* test methods were used. Separated vagal and sympathetic stimulation on the same heart preparation showed that only vagal stimulation yielded thiamine in the perfusion fluid. This perfusion fluid, transferred to another frog heart (after inactivation of the acetylcholine by cholinesterase) showed an inhibiting effect on the action of vagus stimulation on the acetylcholine action of this heart. If the perfusion fluid was irradiated for only 5 minutes by short wave ultraviolet light, the inhibition disappeared because of the photochemical destruction of thiamine. Fig. 10 shows the result of such an experiment by Cottier (unpublished). The same disappearance of the thiamine effect was also observed after treatment of the perfusion fluid with a small amount of Frankonit (thiamine absorbent). Frog experiments are convincing only if confirmed by experiments on warm blooded animals. Gross (1947) has studied the effect of vagal stimulation in the rabbit with and without simultaneous action of thiamine. He found that the injection of thiamine at once decreased the effect of the vagal stimulation and produced a shift in the balance between vagal and sympathetic effects toward the sympathetic side in the heart. Fig. 11 gives an example of the interesting results which he obtained.

Without putting too much stress on an experiment which was performed by the members of the Berne institute, it may be mentioned here

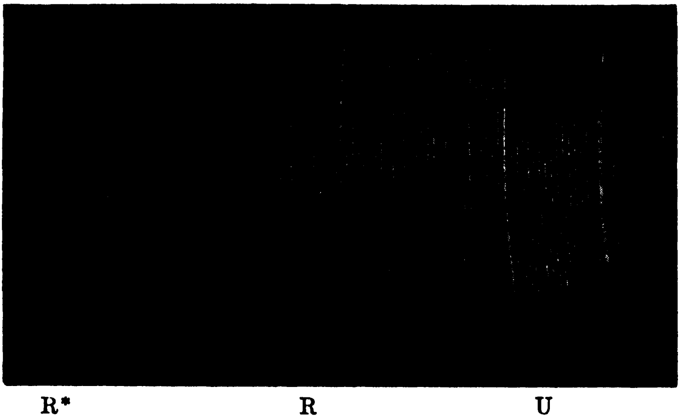


Fig. 10.—Photochemical action of ultraviolet light on a perfusion fluid from vagal stimulation. R* = 1 cc. of a perfusion fluid from a vagal stimulation period, *irradiated;* R = 1 cc. of a perfusion fluid from a vagal stimulation period, *normal;* U = 1 cc. of a perfusion fluid from an unstimulated heart. To all solutions equal amounts of acetylcholine were added.

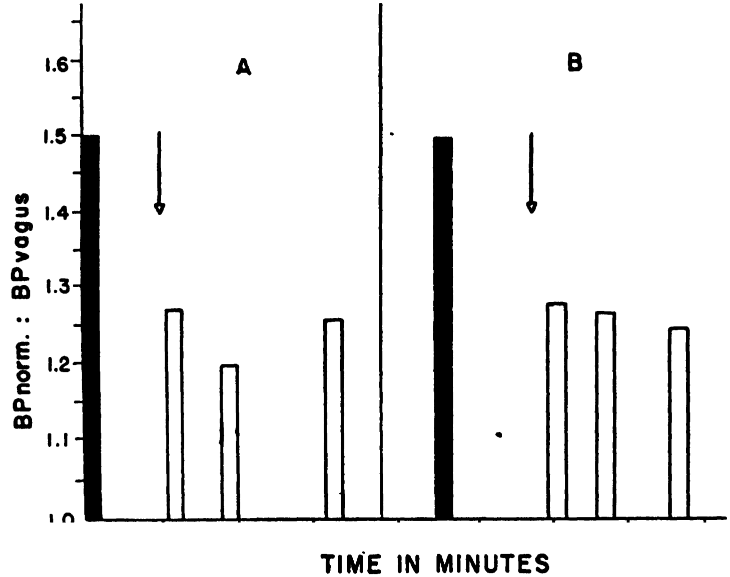


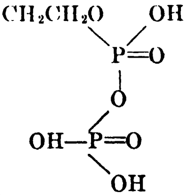
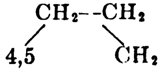
Fig. 11.—Response of blood pressure of the rabbit on vagus stimulation before and after injection of thiamine. Ordinate: Relation of normal blood pressure to minimal blood pressure *during* vagus stimulation; abscissa: time. The arrow in experiment A indicates injection of 1 mg. thiamine/kg. of weight; that in experiment B indicates injection of 2 mg. thiamine/kg. of weight. Note inhibition of the effect of vagus stimulation after injection.

that they made careful observations on the pulse rate at the moment of waking every morning. After having observed their normal average pulse rate for some time, they spent 36 hours without food. This fasting period produced in all subjects a more or less pronounced bradycardia. Fourteen days later the same experiment was performed after injection of thiamine and some subjects reacted with a less pronounced bradycardia on fasting, some without any change of the resting pulse rate, and one showed a small increase.

The inhibiting effect of thiamine on the vagus or acetylcholine action in the heart is not specific. Table IV shows the extent to which thiamine derivatives are active if certain groups in the molecule are replaced by other groups.

TABLE IV

Inhibitory Effect of Thiamine Derivatives on the Action of Acetylcholine on the Heart

Pyrimidin		Thiazol			Activity	Substance
2	4	2	4	5		
CH_3	NH_2	H	CH_3	CH_2CH_2OH	+++	Thiamine chloride
CH_3	NH_2	H	CH_3		– (!)	Cocarboxylase
CH_3	NH_2	H	CH_3	$CH_2CH_2OCOCH_3$	0	Acetylthiamine
CH_3	NH_2	H	CH_3	CH_2CH_2Br	++	5-β-Bromo-ethylester
CH_3	NH_2	H	CH_3	CH_2CONH_2	+	5-Acetic acid amide
C_2H_5	NH_2	H	CH_3	CH_2CH_2OH	+	2-Ethylpyrinidil
CH_3	NH_2	H	. . .		0	4,5-Cyclopenteno-thiazoliumchloride
3-(4,5)-Methylimidazolyl		H	CH_3	CH_2CH_2OH	++	
3-β-Acetoxyethyl		H	CH_3	H	+	Acetoxyethyl-thiazole
				CH_2CH_2OH	......	Thiazole

It is most amazing that cocarboxylase has no action at all. Gross (1946) found that the active water-soluble principle of adrenal cortex, "percorten," has exactly the same action on the heart as thiamine. It is possible that tests on other substances will show them to have the same inhibitory action.

Before Loewi (1937) understood the exact nature of the chemical mediator in the heart, he called it "Vagusstoff." Later it was proven to be acetylcholine. There is no doubt that on vagal stimulation a second substance with inhibitory properties is liberated. This substance so far shows all the reactions of thiamine, but there is insufficient evidence to indicate whether it is thiamine or one of the possible thiamine compounds. As long as this uncertainty exists, Muralt (1945) proposed the substance be called "2. Vagusstoff."

It is worthwhile to mention that thiamine sensitizes the leech preparation toward acetylcholine. It is not improbable that this is one of the reasons why the liberation of acetylcholine in synapses and motor nerve endings, *etc.*, was so easily detected with the leech preparation. This assumption includes the speculation that thiamine might be liberated not only at the endings of the vagus, but at the terminals of all cholinergic nerves. This far-reaching conclusion remains to be proven by experiment.

V. Discussion and Summary

The reader will have been surprised not to find any allusion to the enormous literature on vitamin deficiency effects in relation to the neurophysiological problem. It is the conviction of the reviewer that such experiments cannot teach us more than the very obvious fact that thiamine is important for the mechanism of nerve function. Because it is of such importance, the nervous tissue is one of the last tissues to lose its thiamine content when a state of avitaminosis prevails. When it is losing its thiamine, the general disarrangement in the body is so far advanced that it is hard to distinguish between specific and general effects. There has been a tendency in recent years to ascribe the nervous symptoms observed in avitaminosis to starvation, rather than lack of thiamine. Starvation produces the same sort of peripheral degeneration as thiamine deficiency (Chamberlain *et al.*, 1911). Engel and Phillips (1938) even went so far as to say that the nervous degeneration is due to lack of dietary factors *other* than thiamine! Swank and Prados (1942), however, have shown convincingly that there is a causative connection between the neurological lesions and thiamine deficiency. It is the impaired function which is the first sign, and only then degeneration begins in the nerves. It should be kept in mind, however, that lack of thiamine has a serious effect on the general metabolism of other tissues, and leads to the accumulation of pyruvic acid, and that many effects observed in avitaminosis must thus be ascribed to these changes. Lewy (1937) made chronaximetric determinations in cases of human avitaminosis and saw that the increased excitability (which corresponds to the effects found by Hutton-Rudolph (1944) on irradiating single nerve fibers of the frog) corresponded with the severity of the condition and that the improvement resulting from thiamine therapy could be followed directly by chronaximetric determinations. These indications are sufficient to prove that thiamine is important in nerve metabolism.

What is the function of thiamine in the mechanism of nervous excitation? Insight as to the possible action of thiamine is based on Peters' (1940) classical experiments. From these we know that one mole of cocarboxylase is able to catalyze 1500 moles of oxygen/min. in the pyruvic acid cycle, and that cocarboxylase is a catalytic agent in a number of reactions in which pyruvic acid is the substrate (pyruvodehydrogenase, carboxylase). It is also essential in the resynthesis of carbohydrate from pyruvic acid and lactic acid (Barron and Lyman, 1940; 1941) and has, therefore, an important role in those reactions which in muscle chemistry are known to restore the energy spent in contraction. One must be careful in applying results which are valid in muscle chemistry to the peripheral nerve, but it is always helpful as long as we know so little about the chemistry of the nerve to reason from the known.

Why thiamine is liberated in the normal excitation process and why it is accumulated at rest in monoiodoacetic acid-poisoned nerves and disappears on excitation, is a problem which needs further experimental work. It may be related to the reaction: adenosine triphosphate + thiamine = adenylic acid + cocarboxylase. It has been suggested that this mechanism is essential for the formation of acetylcholine (Muralt, 1943). The scheme which was visualized is the following: The breakdown of adenosine triphosphate activates thiamine to cocarboxylase, which catalyzes the anaerobic and aerobic decarboxylation of pyruvic acid, in the following two ways:

(1) anaerobic

$$2CH_3{\cdot}CO{\cdot}COOH + H_2O = CH_3{\cdot}COOH + CH_3{\cdot}CHOH{\cdot}COOH + CO_2$$

(2) aerobic

$$2CH_3{\cdot}CO{\cdot}COOH + O_2 = 2CH_3{\cdot}COOH + CO_2$$

These reactions furnish the acetic acid necessary for the acetylation of choline, derived from the dephosphorylation of nerve phosphatides. Adenosine triphosphate and cocarboxylase act as phosphate donor and acceptor, and are connected with the degradation of glucose, acting as energy transmitters.

Nachmansohn (1945), without knowledge of Muralt's papers, because of the war, independently gave a scheme which is based on the same general idea and largely extended to the acetylcholine-phosphocreatinside, but did not include the thiamine-cocarboxylase-adenylic acid aspect. He mentions, however, that the acetyl portion of acetylcholine is derived from pyruvic acid and that cocarboxylase plays an important role in the oxidation of pyruvic acid. Nachmansohn and Steinbach (1942) found that the vitamin, determined as diphosphothiamine, is concentrated at the surface of the axoplasm and, therefore, is close to the region where acetylcholine metabolism takes place. It may be mentioned here that this quite independent and important approach, based on studies of cholinesterase in nerve, led Nachmansohn (1945) to propose the same concept of the role of acetycholine liberation along the axone and across the synapse, as was expressed by Muralt (1942) on the basis of his freezing experiments.

Thus far thiamine and cocarboxylase have been considered as catalysts. This view does not correspond to the rather large daily requirement of thiamine in the body. Thiamine must also be considered as a metabolic substance. How thiamine or thiamine compounds could participate directly in the metabolism of the nerve, besides functioning as a catalyst, is a question which cannot easily be answered on the basis of present knowledge.

This review has given proof that thiamine plays an important role in neurophysiology, a role which can only be explained on the basis of further experimental work.

REFERENCES

1. Agid, R. and Balkanyi, J. 1938. *Compt. rend. soc. biol.* **127,** 680.
2. Atkin, L., Schultz, A. S., and Frey, C. N. 1939. *J. Biol. Chem.* **129,** 471.
3. Barron, E. S. G., and Lyman, C. M. 1940. *Science* **92,** 337.
4. Barron, E. S. G., and Lyman, C. M. 1941. *J. Biol. Chem.* **141,** 951, 957.
5. Bergami, G. 1936a. *Boll. soc. ital. biol. sper.* **11,** 275.
6. Bergami, G. 1936b. *Arch. inst. biochim. ital.* **2,** 130.
7. Bicknell, F., and Prescott, F. 1946. The Vitamins in Medicine, 2nd ed., William Heinemann, London.
8. Binet, L., and Minz, B. 1934. *Compt. rend. soc. biol.* **117,** 1029.
9. Brown, G. L., and Feldberg, W. 1936. *J. Physiol.* **88,** 265.
10. Brücke, F. v., and Sarkander, H. 1940. *Arch. exptl. Path. Pharmakol.* **195,** 218.
11. Calabro, Q. 1933. *Riv. Biol.* **15,** 299.
12. Caspersson, T. 1940a. *J. Roy. Microscop. Soc.* **60,** 8.
13. Caspersson, T. 1940b. *Chromosoma* **1,** 562.
14. Chamberlain, W. P. 1911. Cited by Bicknell and Prescott, 1946.
15. Dale, H. H. 1935. Reizübertragung durch chemische Mittel im peripheren Nervensystem. Urban and Schwarzenberg, Vienna.
16. De Caro, L., and Butturini, L. 1940. *Boll. soc. ital. biol. sper.* **15,** 406.
17. Eijkman, C. 1897. *Arch. path. Anat.* (*Virchow's*) **148,** 523; **149,** 187.
18. Engel, R. W., and Phillips, P. H. 1938. *J. Nutrition* **16,** 585.
19. Feldberg, W. 1943. *J. Physiol.* **101,** 432.
20. Gaddum, J. H., Khayyal, M. A., and Rydin, H. 1937. *J. Physiol.* **89,** 9.
21. Grijns, G. 1901. *Tijdschr. Ned. Ind.* **41,** 3.
22. Gross, F. 1946. *Experientia* **2,** 191.

22a. Gross, F. and Strauss. 1947. *Helv. Physiol. Acta* **5**, 137.
23. Holter, H., and Linderström-Lang, K. C. 1943. *Compt. rend. trav. lab. Carlsberg* **24**, 17/18.
24. Hutton-Rudolph, M. 1943. *Helv. Physiol. Acta* **1**, C15.
25. Hutton-Rudolph, M. 1944. Dissertation, Berne.
26. Jansen, B. C. P. 1936. *Rec. trav. chim.* **55**, 1046.
27. McIntosh, F. C. 1938. *Arch. intern. physiol.* **47**, 312.
28. McIntosh, F. C. 1941. *J. Physiol.* **99**, 436.
29. Kaiser, P. 1939. *Arch. ges. Physiol. (Pflüger's)* **242**, 504.
30. Kuhn, R., Wagner-Jauregg, T., v. Klaveren, F., and Vetter, H. 1935. *Z. physiol. Chem.* **234**, 196.
31. Lewy, F. H. 1937. *J. Clin. Invest.* **16**, 475.
32. Liechti, A., Muralt, A. v., and Reinert, M. 1943. *Helv. Physiol. Acta* **1**, 79.
33. Loewi, O. 1937. Die chemische Uebertragung der Nervenwirkung. Nobel-Comité, Stockholm.
34. Lüthy, H. 1946a. *Helv. Physiol. Acta* **4**, C20.
35. Lüthy, H. 1946b. *Helv Physiol. Acta* **4**, C50.
36. Minz, B. 1938a. *Compt. rend. soc. biol.* **127**, 1251.
37. Minz, B. 1938b. *Presse méd.* **76**, 1406.
38. Moniz de Bettencourt, J., and Paes, E. 1936a. *Compt. rend. soc. biol.* **122**, 237.
39. Moniz de Bettencourt, H., and Paes, E. 1936b. *Arch. port. sci. biol.* **5**, 133.
40. Muralt, A. v. 1937. *Proc. Roy. Soc. London.* **123**, 399.
41. Muralt, A. v. 1939a. *Sitzber. physik.-med. Sozietät Erlangen* **71**, 175.
42. Muralt, A. v. 1939b. *Forschungen Fortschr.* **15**, 121.
43. Muralt, A. v. 1939c. *Naturwissenschaften* **27**, 265.
44. Muralt, A. v. 1941. *Verhandl. schweiz. Physiol.* (July).
45. Muralt, A. v. 1942. *Arch. ges. Physiol. (Pflüger's)* **245**, 604.
46. Muralt, A. v. 1943a. *Arch. ges. Physiol. (Pflüger's)* **247**, 1.
47. Muralt, A. v. 1943b. *Schweiz. med. Wochschr.* **73**, 110.
48. Muralt, A. v. 1944. *Schweiz. med. Wochschr.* **74**, 643.
49. Muralt, A. v. 1945. *Experientia* **1**, 136.
50. Muralt, A. v. 1946. Die Signalübermittlung im Nerven. Birkhäuser, Basel.
51. Muralt, A. v., Lotmar, W., and Wilbrandt, W. 1938. *Kongressber. XVI. intern. physiol. Kongr.* 24.
52. Muralt, A. v., and Schulthess, G. v. 1944. *Helv. Physiol. Acta* **2**, 435.
53. Muralt, A. v., and Wyss, F. 1944. *Helv. Physiol. Acta* **2**, 445.
54. Muralt, A. v., and Zemp, J. 1943. *Arch. ges. Physiol. (Pflüger's)* **246**, 746.
55. Muralt, G. v. 1947. Dissertation, Bern.
56. Nachmansohn, D. 1945. *Vitamins & Hormones* **3**, 337.
57. Nachmansohn, D., and Steinbach, H. B. 1942. *J. Neurophysiol.* **5**, 109.
58. Norberg, B. 1942. *Acta Physiol. Scand., Suppl.* **16**.
59. Peters, R. A. 1940. *Nature* **146**, 387.
60. Ritsert, K. 1938. *Klin. Wochschr.* **17**, 1397.
61. Ritsert, K. 1939. *Klin. Wochschr.* **18**, 1370.
62. Rossine, J. A. 1936a. *Bull. biol. méd. exp. U.R.S.S.* **1**, 164.
63. Rossine, J. A. 1936b. *Bull. biol. méd. exp. U.R.S.S.* **2**, 130.
64. Ruehle, A. E. J. 1939. *J. Am. Chem. Soc.* **57**, 1887.
65. Sanz, M. 1943. *Arch. ges. Physiol. (Pflüger's)* **247**, 317.
66. Schmitt, F. O. 1939a. *Biol. Rev. Cambridge Phil. Soc.* **14**, 27.
67. Schmitt, F. O. 1939b. *Physiol. Revs.* **19**, 270.

68. Stämpfli, R. 1942. Dissertation, Bern.
69. Swank, R. L., and Prados, M. 1942. *Arch. Neurol. Psychiat.* **47,** 97.
70. Tower, S. S. 1939. *Physiol. Revs.* **19,** 1.
71. Uber, F. M., and Verbrugge, F. 1940a. *J. Biol. Chem.* **134,** 273.
72. Uber, F. M., and Verbrugge, F. 1940b. *J. Biol. Chem.* **136,** 81.
73. Weidmann, S. 1945. *Experientia* **1,** 61.
74. Weidmann, S. 1946. Dissertation, Bern.
75. Went, L. N. 1946. *Helv. Physiol. Acta,* **4,** C4.
76. Westenbrink, H. G. K. 1940. *Enzymologia* **8,** 97.
77. Westenbrink, H. G. K., and Goudsmit, J. 1938. *Nature* **142,** 150.
78. Wiedenbauer, F. 1939. *Klin. Wochschr.* **18,** 1613.
79. Williams, R. D., Mason, H. L., Wilder, R. M., and Smith, B. F. 1940. *Arch. Internal Med.* **66,** 785.
80. Wyss, A., and Wyss, F. 1945a. *Helv. Physiol. Acta* **3,** C30.
81. Wyss, A., and Wyss, F. 1945b. *Experientia* **1,** 160.
82. Wyss, F. 1943. *Helv. Physiol. Acta* **1,** C70.
83. Wyss, F. 1944. *Helv. Physiol. Acta* **2,** 121.
84. Wyss, F., and Muralt, A. v. 1944. *Helv. Physiol. Acta* **2,** C61.

The Physiological Effects of the Pteroylglutamates in Man—with Particular Reference to Pteroylglutamic Acid (PGA)[1]

By WILLIAM J. DARBY

CONTENTS

Page

I. Introduction 119
   1. Nomenclature 120
   2. Historical 122
II. Sprue 124
   1. Clinical Description 124
   2. Effects of Pteroylglutamates 125
      a. Experimental Background 125
      b. Clinical and Hematological Effects 126
      c. Effects on the Gastrointestinal Tract 131
   3. Estimated Daily Requirements of Vitamin M 136
III. Nutritional Macrocytic Anemia 139
IV. Macrocytic Anemia of Pregnancy (Pernicious Anemia of Pregnancy) . . . 140
V. Pernicious Anemia 141
   1. Clinical Description 141
   2. Effects of Pteroylglutamates 142
      a. General Clinical 142
      b. Reticulocyte Response 144
      c. Erythrocyte Response 147
      d. Other Effects 149
   3. Evidence Relative to the Nature of a Metabolic Defect 150
VI. Pteroylglutamates in the Treatment of Diseases of Infancy and Childhood 152
VII. Pteroylglutamates in the Treatment of Chronic Diarrheas 154
VIII. Thymine 154
IX. A Resumé of the Nutritional Physiology of Vitamin M in Man 155
References 157

I. INTRODUCTION

The convergence of separate paths of investigation has occurred with the synthesis of pteroylglutamic acid (Angier *et al.*, 1945, 1946). The animal nutritionist, the microbiologist, the clinical investigator, and the organic chemist have again met on a common road. It is not the purpose of the present review to map the paths by which these investigators have come. Rather, certain of the recently passed milestones will be noted and the reader will be referred to other reviews for the details of the individual routes.

This review describes the known physiological effects in man of this newest vitamin, summarizes the existing data on its therapeutic usefulness, and points to the unknown areas of our knowledge. No attempt is made to reinterpret the earlier work in which unidentified concentrates have been used that may or may not have contained members of this family of substances. Pfiffner and Hogan (1946) have written on this subject in a previous volume of this publication. Every effort is made in this discussion to identify each member of the group, but such identification is not always possible from the meager descriptions provided in some of the reports. Where the latter situation prevails, the reviewer has been forced to employ the same loose terminology as the original author. The literature survey upon which the review is based is complete through those journals received prior to September 26, 1946. In addition, through the generous cooperation of several independent investigators it has been possible to incorporate a rather large body of unpublished data, a fact which has facilitated the evaluation of some of the therapeutic activities of the vitamin. I am most grateful to those investigators who have permitted me to employ their data in this manner. Since the initial preparation of the manuscript some of the unpublished data have appeared. These literature citations have been added in press.

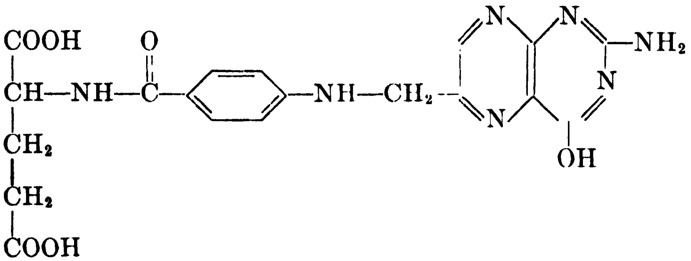
---

[1] This review is based on a paper presented at the AAAS Conference on Vitamins, Gibson Island, July 29–August 2, 1946.

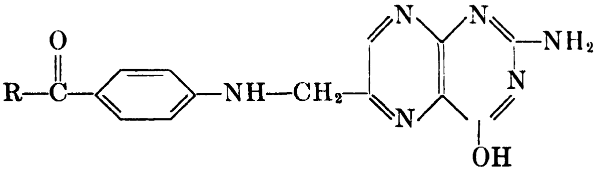
1. *Nomenclature*

It may be justifiable to review briefly the nomenclature of this family of vitamins and growth substances in order to orient the relationships. The first definitive name was "vitamin M" which was suggested in 1938 (Day, Langston, and Darby, 1938; Langston *et al.*, 1938) for a previously undifferentiated factor which prevents nutritional cytopenia in the monkey. The same year Stokstad and Manning (1938) used the term "factor U," and the following year Hogan and Parrott (1939, 1940) applied the designation "vitamin B_c" to factor(s) required by the chick and later isolated from liver (Pfiffner *et al.*, 1943). Snell and Peterson (1939, 1940) referred to a substance necessary for *L. casei* as the "Norit eluate factor," following which Mitchell, Snell, and Williams (1941) named a similar growth substance required by *S. lactis R* (*S. faecalis*) "folic acid." Stokstad (1943) prepared two growth factors required by *Lactobacillus casei* and these were termed "*L. casei* factors." One was obtained from yeast, the other from liver. A third "*L. casei* factor," later known as "fermentation *L. casei* factor," was reported as isolated from a fermentation residue (Hutchings *et al.*, 1944). The source of this compound has been revealed to be a diphtheroid-like organism (Hutchings and collaborators, 1946). Mims, Totter, and Day (1944) differentiated a "potential" folic acid in foodstuffs and Bird *et al.* (1945) applied the term "vitamin B_c conjugate" to an obviously similar material (Binkley *et al.*, 1944) which they later isolated from yeast (Pfiffner *et al.*, 1945). A number of reviews have summarized the experimental evidences of relationships between these dietary and growth essentials (Anonymous, 1944, 1945, 1946; Day, 1944; Hogan, 1944; Wieder, 1944; Daft and Sebrell, 1945; Berry and Spies, 1946; Day and Totter, 1947; Norris and Heuser, 1945; Totter, 1945; Pfiffner and Hogan, 1946).

Pteroylglutamic acid, which may conveniently be referred to as PGA, was synthesized by Angier *et al.* (1945) and the structure later revealed (Angier *et al.*, 1946) to be I. The moiety containing the pterin and the *p*-aminobenzoic acid was named pteroic acid (II, R = OH) and the acyl radical was termed the pteroyl radical. Pteroylglutamic acid has proved to be a fundamental unit common to a family of biologically active substances which may be termed pteroylglutamates. These differ in the number of glutamic acid residues in the side-chain, R. Thus, pteroyltriglutamic acid (R = three glutamic residues) is the fermentation factor of Hutchings *et al.* (1944) (Angier, *et al.*, 1946), and pteroylheptaglutamate (pteroylhexaglutamylglutamic acid) is the substance previously designated as vitamin B_c conjugate (Pfiffner *et al.*, 1946).



I



II

PGA is identical with liver *L. casei* factor and with vitamin B_c (Johnson, 1946; Pfiffner *et al.*, 1946); it is biologically active for the chick, for *L. casei* and *S. faecalis* (Angier *et al.*, 1945), the sulfa-treated rat (Daft, 1946), for the monkey (Cooperman *et al.*, 1946; Day and Totter, 1946; Saslaw *et al.*, 1946), and for man (*vide infra*). The varying biological activity of these compounds makes the group name "folic acid" untenable. The chemical designation of "pteroyl derivatives" or "pteroylglutamates" should be adopted for the chemical family. The term "vitamin M group" should also be useful as a designation for those members of the group chemically related to PGA and which exhibit hemopoietic activity for primates (Darby, Jones, and Johnson, 1946b). The identity of the members of the vitamin M group has not yet been established; however, pteroylglutamic acid (Cooperman *et al.*, 1946), pteroyltriglutamic acid (Day *et al.*, 1945; Day, Mims, and Totter, 1945), and pteroylheptaglutamic acid (Cooperman *et al.*, 1946) all appear to be active for the monkey. The same three compounds are hemapoietically active in sprue (Darby and Jones, 1945a; Moore *et al.*, 1945; Suárez, Welch *et al.*, 1946; Jones, Warden, and Darby, 1947); the monoglutamate and triglutamate are active in nutritional macrocytic anemia (*q.v.*); and at least PGA is active in pernicious anemia (*q.v.*).

2. Historical

Prior to the use of synthetic PGA the most readily available material of this group was the fermentation factor of Hutchings *et al.* (1944). This substance was tested by Castle *et al.* (1944) in two cases of pernicious anemia and was found to be inactive in doses of 2.3 and 3.6 mg. daily by mouth over a ten-day period. Sharp and Vonder Heide (1944) reported that concentrates of vitamin B_c made from yeast and liver were inactive in 6 patients with pernicious anemia in relapse in oral doses ranging from 0.44 to 1.5 mg. daily. Sharp, Vonder Heide, and Wolter (1944) had previously recorded the inactivity of a vitamin B_c concentrate from yeast in 10 cases of refractory anemia. Watson *et al.* (1945) obtained similar negative results in 8 cases of refractory anemia to which they administered fermentation *L. casei* factor in oral daily doses of 5 mg. The latter investigators, however, did obtain encouraging results with the substance in 6 cases of leucopenia following Roentgen-ray therapy. They concluded that their findings warranted further study of the possible effectiveness of *L. casei* factor in leucopenia, particularly that following radiation. Finally, Berry, Spies, and Doan (1945) likened the leucopenia which they sometimes observed in malnourished patients to the leucopenia which is part of the nutritional cytopenia in the vitamin M-deficient monkey, and which responds to treatment with vitamin M. They, therefore, injected 17 patients with a "special fraction of liver extract containing, among other things, folic acid," and observed that 12 of the patients showed an increase of from 23 to 130% in the total leucocyte count, sometimes within a period of 2.5–5 hours after the injection. Similar responses occurred in 5 patients who were treated with "crystalline *L. casei* factor," 15–20 mg. each. These studies are difficult to evaluate inasmuch as the data extend over only a few hours and there were reported no controlled studies of the effect of injection of the material in healthy individuals. It was stated, however, that additional injections were given at weekly intervals for periods of from 1–5 months and that the higher leucocyte counts were maintained in the majority of cases. Of the five cases which failed to exhibit the response to the liver concentrate it is of interest that one was described as a macrocytic anemia in relapse. It is of further interest that this report in September, 1945, did not mention the similarity of the other aspects of vitamin M deficiency in the monkey to any of the clinical syndromes with which it has since been related. Spies (1946c) later stated that it was the response of patients in this study which suggested to him that "folic acid was probably an extremely important factor in red-blood-cell regeneration."

During the period of slightly over a year since the synthetic material has been available for clinical investigation there have appeared over 50 reports of the effects of this vitamin on man and studies of its metabolism. The quantity of these reports is hardly a measure of their quality. Most of the clinical reports have been brief and contain few data and there is great duplication of cases in them. This rapid flood of papers was occasioned by the variety of diseases affected by the vitamin.

In November, 1945, there appeared a preliminary report (Darby and Jones, 1945a) from Vanderbilt that this substance was active in small amounts in treating the anemia and absorptive defect in sprue and a report from Birmingham (Spies *et al.*, 1945) that it had proved effective in producing remissions in 9 cases of unclassified macrocytic anemia. The following month the Birmingham group (Vilter, Spies, and Koch, 1945) classified their previously published cases as (*a*) nutritional macrocytic anemia, (*b*) Addisonian pernicious anemia, and (*c*) indeterminate. The same report carried a footnote which confirmed our finding that sprue responded to this factor. The footnote stated that " . . . the macrocytic anemia of pernicious anemia, pellagra, sprue, and pregnancy practically all respond to either parenteral or oral administration of synthetic folic acid." This same month, December, Moore *et al.* (1945), in St. Louis, reported complete hematologic data on 2 cases of pernicious anemia, one case of sprue, and a case of pernicious anemia of pregnancy, all of which had responded to the synthetic PGA. In February, Zuelzer and Ogden (1946a) announced that a macrocytic anemia of infants responded to this factor and Doan, Wilson, and Wright (1946) reported that 3 patients with pernicious anemia, and who were sensitive to liver extract, had been successfully treated with *L. casei* factor without allergic reactions. Finally, in March, Spies *et al.* (1946a) reported that a case of pernicious anemia had responded to very large quantities of thymine. Most of these reports have dealt with the hemapoietic activity of PGA, but certain of them, particularly those from our clinic and laboratory, have indicated that the material is necessary for maintaining the normal absorptive function of the gastrointestinal tract.

Since the most varied and profound effects of PGA are illustrated by the studies of patients with sprue, the different aspects of this disease will be discussed first.

II. SPRUE[2]

Sprue is a clinical syndrome first differentiated almost 200 years ago. The relative roles of infection or alteration in the flora of the gastrointestinal tract and of dietary deficiency in this disease have been disputed, but since the extensive studies of Castle *et al.* (1935) it is widely accepted that the syndrome is a deficiency state which can be alleviated by a substance present in liver and liver extracts effective in treating pernicious anemia. Claims of the effectiveness in sprue of first one known vitamin and another have been advanced, but none of these were substantiated. It is generally agreed that the classification into tropical and non-tropical sprue is not justified by any basic difference in the disease in the two areas (Hanes, 1946; Barker, 1943).

1. *Clinical Description*

This disease is characterized by glossitis, diarrhea with increased fat content of the feces (steatorrhea), loss of body weight, pigmentation of the skin, and macrocytic anemia accompanied by moderate leucopenia. The bone marrow exhibits hyperplasia of the erythrocytic series with many megaloblasts. On X-ray examination of the gastrointestinal tract following a barium meal there is usually observed the so-called "deficiency pattern" with smoothing out of the contour of the bowel, some dilation of the gut, and clumping of the barium in irregular masses. There may be hypermotility of the gut. Free hydrochloric acid in the stomach contents, although often decreased in amount, was present in 65% of Hanes' (1946) series. *Absorption from the gastrointestinal tract is impaired* as indicated by a flat oral glucose tolerance curve with a normal intravenous curve, the failure of blood phosphorus to rise after oral glucose, an increased fecal fat accompanying an increased intake and a flat vitamin A tolerance curve, and decreased lipid tolerance. The wasting of the body itself, in the absence of an increased metabolism and (sometimes) in the presence of a quantitatively satisfactory food intake, reflects a caloric loss due to poor absorption. There may occur many evidences of secondary deficiencies of fat-soluble vitamins—nightblindness due to vitamin A lack, the hypoprothrombinemia of vitamin K deficiency, and hypocalcemia, possibly in part due to vitamin D deficiency.

---

[2] For summaries of and references to the clinical studies on sprue see Castle *et al.* (1935), Castle and Minot (1936), Wintrobe (1946), Hurst (1942), Barker (1943), Hanes (1946), and Ingelfinger (1943).

These changes are usually reversible following therapy with liver. The earliest changes after treatment are an improved sense of well-being, disappearance of glossitis, evidence of increased hemapoiesis, and increased absorption of water-soluble substances (evidenced by the return toward normal of the glucose tolerance curve). The diarrhea ceases, but the stools may contain abnormally large quantities of fat for a longer period of time, a phenomenon reflected by the slow rise in fat-soluble vitamins in the blood. The gastrointestinal pattern returns to normal slowly, if at all. The pigmentation of the skin clears. Some cases of sprue of long standing may show incomplete responses to massive liver therapy.

2. *Effect of PGA*

a. Experimental Background. In November, 1945, the Vanderbilt group announced the rapid response of three cases of sprue following the intramuscular administration of 15 mg. of PGA daily (Darby and Jones, 1945a,b; Jones, 1945; Darby, Jones, and Johnson, 1946a). The detailed data on these three cases have been presented (Darby, Jones, and Johnson, 1946b) and their subsequent courses have been carefully followed. One of these patients has suffered a relapse after the withholding of PGA and a second remission has now been induced with the synthetic vitamin (Darby and Jones, 1946). Subsequent reports (Moore *et al.*, 1945; Spies *et al.*, 1946b,c,d) have confirmed the effectiveness of small quantities of this vitamin in the treatment of patients with sprue. The dosages employed have ranged from 5 mg. to 200 mg. *per os* daily. There appears little doubt that PGA is a vitamin active for man.

A brief summary of the experimental background of vitamin M deficiency which directed our attention to the possibility that sprue in man might be related to vitamin M deficiency may be outlined as follows:

(1) Many species of animals require at least one of the several closely related substances of this group in order to prevent an anemia. The deficiency syndrome in the monkey is characterized by anemia, leucopenia, diarrhea, inconstant stomatitis, weight loss, and death.

(2) A deficiency of a similar, if not identical, factor had been demonstrated by Wills (1934) to be the cause of tropical macrocytic anemia.

(3) Wills, Clutterbuck, and Evans (1937), Wills and Evans (1938), and Napier (1938) had differentiated this latter factor, which Day (1944) considers identical with vitamin M, from the anti-pernicious anemia

substance present in certain purified liver extracts. We had confirmed this finding (Langston *et al.*, 1938) using vitamin M-deficient monkeys. O'Dell and Hogan (1943) had reported that a potent anti-pernicious anemia fraction of liver was inactive as anti-anemic principle for the chick. Finally, Castle *et al.* (1944) had found that doses up to 3.6 mg. daily of the fermentation *L. casei* factor were ineffective in the treatment of patients with pernicious anemia, and Clark (1945) had reported on the very small amounts of *L. casei* factor present in potent liver extracts. Goldsmith (1945), however, had announced that a case of nutritional macrocytic anemia had shown a submaximal reticulocyte response to small amounts of the fermentation *L. casei* factor.

(4) Alterations in the gastrointestinal tract, manifested by diarrhea, oral lesions, and decreased resistance to infection are outstanding features of vitamin M deficiency in the monkey. This part of the syndrome has been remarked upon by all workers since McCarrison's (1921) now prophetic description: "The diarrhoeic motions were small, numerous and pale in colour . . . In animals receiving butter in addition to the autoclaved rice, another feature was added to the microscopical character of the stools; large amounts of fatty acids were present. In such cases the stools were paler than usual, and in two instances distinctly frothy, resembling those of sprue, the resemblance being carried further by their high fatty acid content." Villada (1942) had also remarked on the similarity of this deficiency disease in monkeys to anemia which occurs in man with accompanying gastrointestinal symptoms.

(5) Vitamin M-deficient monkeys responded dramatically to small amounts of highly purified fermentation factor. The response included improvement in both the blood picture and the gastrointestinal manifestations of the deficiency (Day *et al.*, 1945).

These considerations led us to predict that the vitamin M deficiency syndrome in the monkey was the experimental analogue of sprue in man. Therefore, the activity of the synthetic PGA was tested on the first patients with sprue who became available to us. Simultaneously, this substance was being tested in other clinics, Birmingham, Boston, St. Louis, New Orleans, and elsewhere. The backgrounds of each of these groups of investigators were different, and, hence, the type of case initially tested varied.

b. Clinical and Hematological Effects. All of the reports agree as to the clinical response following therapy with this agent. The first effect of PGA in sprue is a subjective sense of well-being which the patient may express by the statement that he feels better and stronger. This occurs within 24–36 hours and, therefore, before improvement is evident in the peripheral blood picture. The patient then notes that his tongue

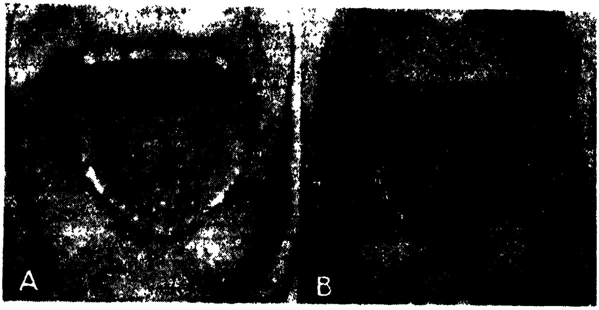


Fig. 1.—The influence of PGA on the glossitis of sprue. (*a*) Tongue of patient prior to therapy, showing red, smooth, glistening surface. (*b*) Tongue of the same patient three weeks after therapy with PGA was instituted. Note the papillary regeneration which had been apparent for some two weeks. (Darby, Jones, and Johnson, 1946b.)

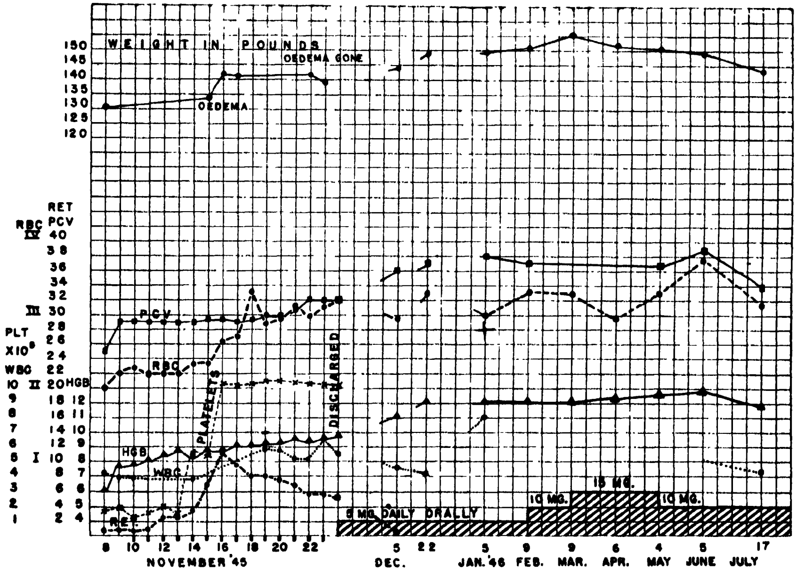


Fig. 2.—Hematologic and weight response of a patient with sprue treated in Vanderbilt University Hospital with PGA. Therapy with 15 mg. daily intramuscularly instituted on November 10. Oral therapy substituted on November 24 in dosages indicated.

is less sore and within 2–4 days the complaint of glossitis disappears. About the fourth day objective evidence of papillary regeneration of the atrophic lingual papillae begins to be manifest and the regeneration continues (Fig. 1). Simultaneously, the number of stools per day decreases and the character of the stools changes from the greasy, semi-solid to watery, yellowish, sometimes frothy, foul-smelling movement to a more nearly normal, darker, formed stool. Spies *et al.* (1946c) have noted that this change is less rapid in the chronic cases, an observation which agrees with our experience. By the third or fourth day the appetite has increased and the patient exhibits an almost insatiable hunger. Simultaneously, a rapid gain in weight occurs, usually accompanied by mild dependent edema (Fig. 2) reflecting an increased water retention in the tissues. The edema may reach its height about the time of the reticulocyte crisis. A similar edema has been noted by Holmes (1945) following the administration of liver extract to patients with macrocytic anemia. Water loss follows, but the weight gain continues. Increased hemapoiesis is evident beginning the third to fifth day and the reticulocyte peak is reached between the fourth and twelfth day (average, seventh day).

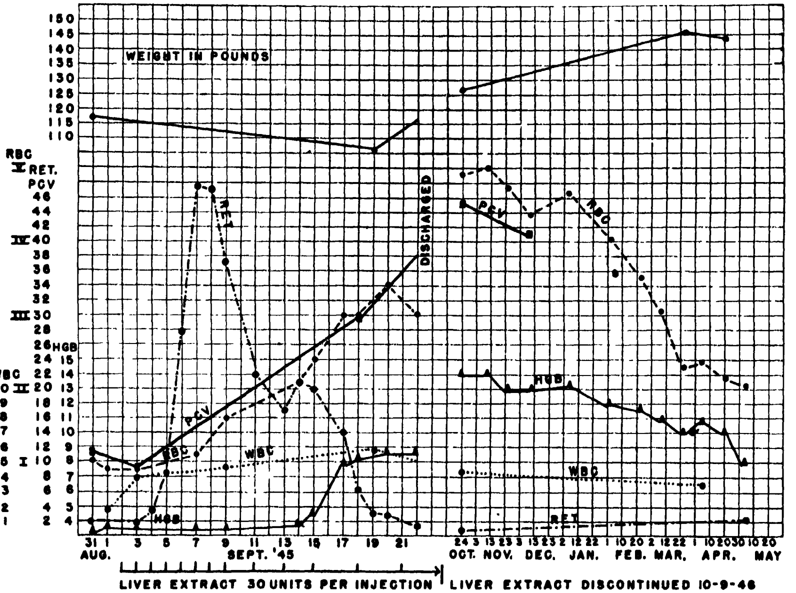


FIG. 3a.—Hematologic and weight response of a patient with sprue treated in the Vanderbilt University Hospital with intramuscular liver extract, 30 units/injection as indicated by vertical bars commencing August 2, 1945. Therapy withdrawn in September with resulting relapse as depicted.

Simultaneously, with the rise in erythrocytes there occurs an outpouring of platelets in the peripheral blood and the bone marrow changes from the hyperplastic, megaloblastic type through a normoblastic marrow (of relative iron deficiency) to a normal marrow as red cell repletion takes place. These hematologic and weight changes are illustrated in Figs. 2 and 3. Figs. 3a and 3b permit the comparison of the response of a patient to intramuscular liver therapy with the response of the same patient to 5 mg. of PGA daily by mouth. It is apparent that the response to the latter agent was as complete and as rapid as was the earlier response to liver extract. Figs. 4a and 4b depict the response of a patient to the parenteral administration of 15 mg. daily of PGA, the continued improvement of the patient for a short period following the withholding of the therapy, the succeeding relapse of the patient, and, finally, the second remission induced by PGA, 5 mg. daily *per os*. It is evident that the second remission of this patient paralleled his previous one despite the difference in size of dose and mode of administration.

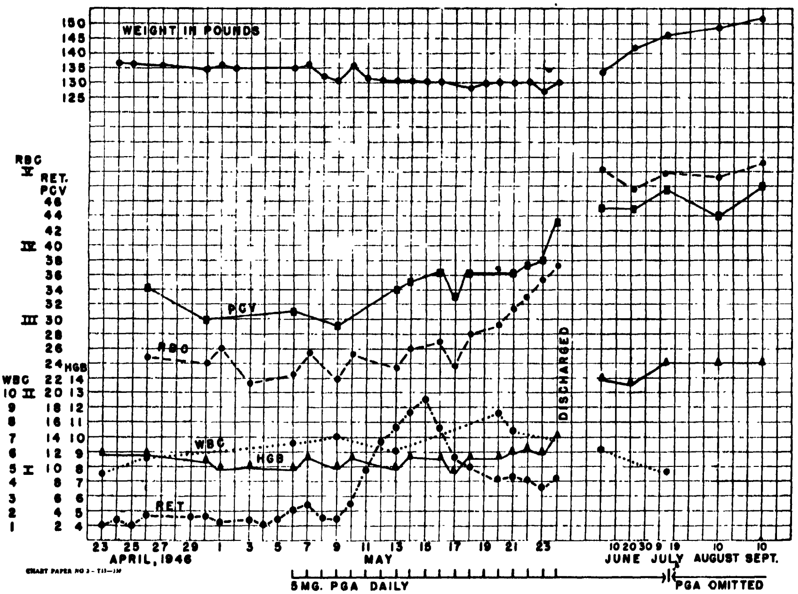


FIG. 3b.—Hematologic and weight response of same patient when treated with 5 mg. PGA orally/day. Therapy instituted April 6. Compare with 3a. The responses to the two agents are very similar and the final hematological levels obtained are identical.

In an attempt to compare the hematologic response of patients with sprue to treatment with PGA with patients treated with liver extract the reticulocyte maxima for patients treated by the two methods are compared in Figs. 5 and 6. The available data indicate that the reticulocyte response of patients with sprue following oral therapy with PGA is greater than that following oral liver extract therapy. Furthermore, the response of sprue patients to PGA follows very closely the expected response of patients with pernicious anemia who are adequately treated with liver extract. This is in contrast to the generally held belief that patients with sprue show less pronounced responses than patients with pernicious anemia, and it may indicate that PGA more completely replaces the deficiency in sprue than does liver extract. This point, however, is by no means established as there are other possible explanations for the observations, such as the use of different staining technics in the counting of reticulocytes. The data available to the reviewer do not permit a categorical statement as to whether the erythrocytes attain as high a level in all cases following the treatment of sprue with PGA as they do after therapy with liver. None of the data clearly indicate that liver extract has produced any greater rise in erythrocytes in sprue than has

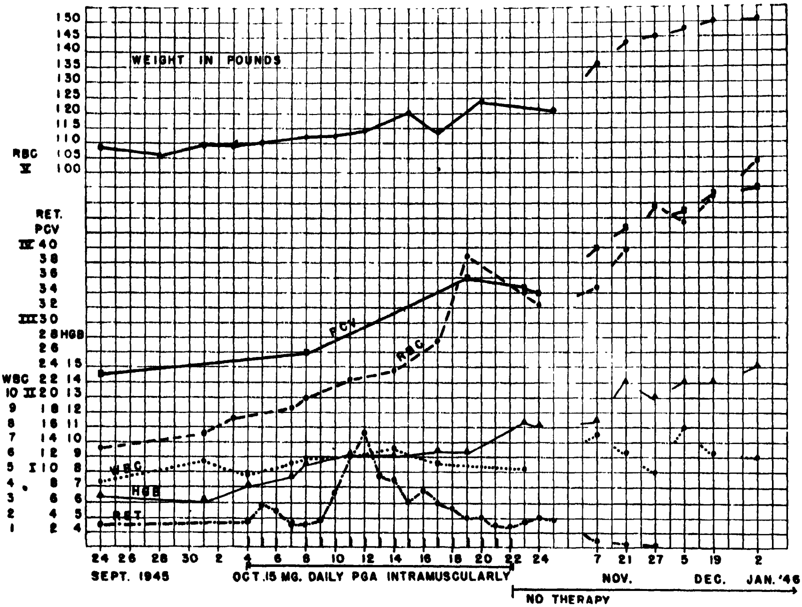


FIG. 4a.—Hematologic and weight response of a patient in Vanderbilt University Hospital with sprue to administration of 15 mg. of PGA intramuscularly/day from October 4 to 22, 1945. Therapy discontinued and the patient's subsequent course is shown. Note the continuation of the increased erythropoiesis after therapy was stopped. This probably indicates storage of the vitamin.

PGA. In our observations (Darby and Jones, 1946c) of five remissions of sprue induced by PGA only one of the patients has failed to attain an erythrocyte level well above four million/mm.[3] This one patient had been treated in previous relapses with very large quantities of liver extract with no greater response than he has exhibited to the synthetic material. The average red blood cell count attained by our four patients (5 relapses) following treatment with PGA is 4.35 million/mm.[3] The macrocytosis in sprue can be reduced by PGA (Table I). The hemoglobin has attained correspondingly satisfactory levels.

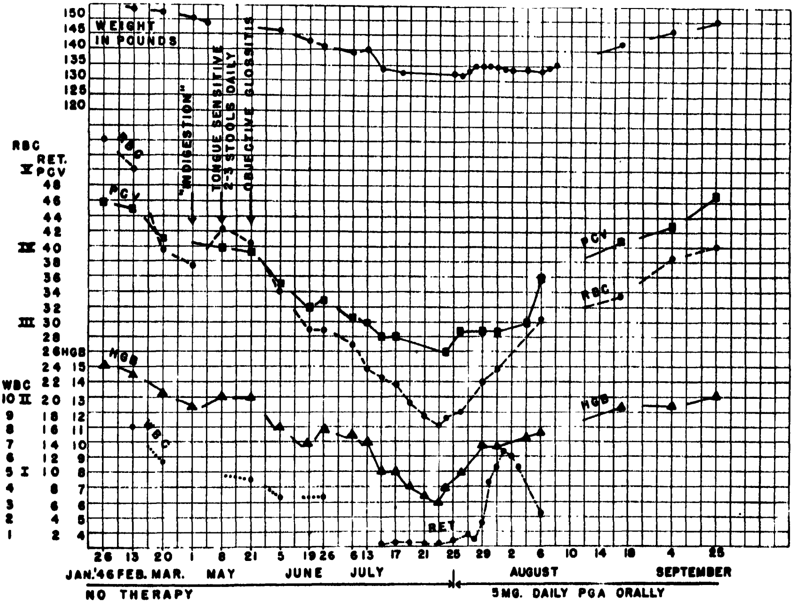


FIG. 4b.—Relapse of patient with sprue (continued from 4a) and remission induced by oral administration of 5 mg. PGA daily beginning July 25. Compare with previous response (4a) to parenterally administered PGA.

c. Effects on the Gastrointestinal Tract. As noted above, the absorptive defects in sprue may not be reversible in every case. However, it is well to examine the evidence, direct and indirect, for an effect of PGA upon gastrointestinal absorption in the disease. Again, the earliest reported effect of the substance on gastrointestinal absorption was by the Vanderbilt investigators who noted a return toward normal of the oral glucose tolerance and vitamin A tolerance curves (Darby and Jones, 1945a,b). The increasing maximum of the glucose tolerance curve following therapy is well illustrated in Fig. 7 and is similar to the effect which followed liver therapy in the same patient several years earlier. Like results have been obtained in other cases (Darby, Jones, and Johnson, 1946b; Darby and Jones, 1946c; Suárez, Spies, and Suárez, 1946).

The weight gains by these patients are often astounding (see Figs. 2, 3, 4, 8) and are undoubtedly due in part to the improved absorption from the gastrointestinal tract. The decreased steatorrhea alone may account for a saving of 600 or more calories per day. The diminished loss of fat in the stools is impressive when expressed as total fat/24 hours rather than as *per cent* of dry weight (Fig. 8). It is well-known that the content of fecal fat in sprue may vary considerably with the quantity of fat in the diet (Black, Fourman, and Trinder, 1946). It is reasonable, therefore, to regard the improved absorption of fat-soluble vitamins as a better indicator of improved absorption of lipides than the occasional determination of fecal fat on an uncontrolled dietary. With any improvement of absorption it is to be expected that the replenishment of fat-

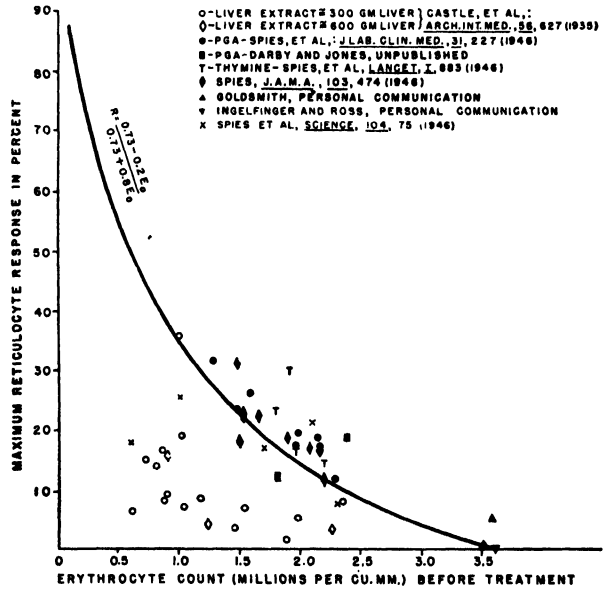


FIG. 5.—Comparison of maximum reticulocyte response in sprue following orally administered liver extract, PGA, or thymine with the expected response of adequately treated cases of pernicious anemia. The curve is that of the indicated formula which was derived by Riddle (1930) to express the average response of patients with pernicious anemia adequately treated with liver extract. Note that the responses of patients with *sprue* treated with PGA or thymine orally cluster about the standard response for pernicious anemia and are higher than the maxima of sprue patients treated with liver extract orally.

soluble vitamins in the blood would be gradual unless massive supplementation with these substances was instituted. We have not provided such supplements for our patients. The data in Table II, therefore, indicate the gradual changes of concentration of fat-soluble factors which occurred in our cases of sprue after therapy with PGA and following the withdrawal of such therapy (Darby, Cherrington, and Ruffin, 1946; Darby, Kaser, and Jones, 1947). Fig. 9 gives the data illustrating the flat vitamin A absorption curves obtained in one of these cases of sprue in relapse, the slight change in the curve during an early remission induced by PGA, the return to an essentially normal curve during a complete remission induced by therapy with PGA, and, finally, a reversion to the flat type of curve during a relapse. These data, together with the somewhat similar changes in tocopherol levels in sprue following therapy (Table II), indicate that PGA can favorably affect the absorptive defects which exist in the disease. Thus, PGA would seem to be interrelated with a number of foodstuffs. One may predict that, as with liver therapy, cases will be encountered in which the absorptive defects are not so readily corrected as in those cited here. This may be expected of the

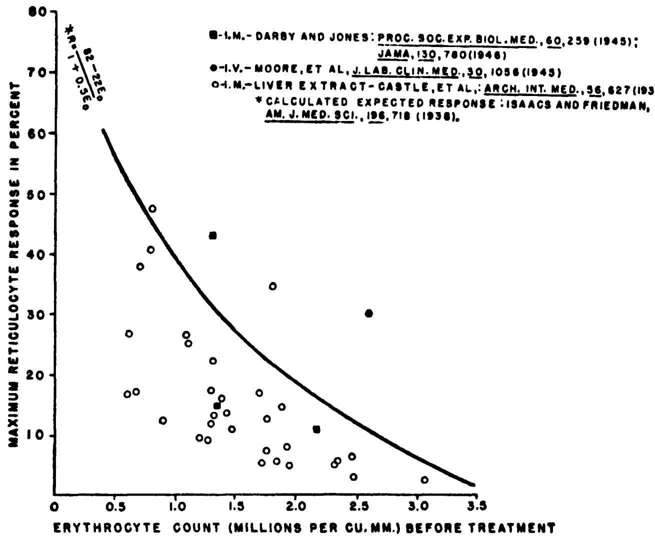


FIG. 6.—Comparison of the maximum reticulocyte response in sprue following therapy with PGA or liver extract parenterally with the expected response of patients with pernicious anemia. The curve is that of the indicated formula which was derived by Isaacs and Friedman (1938) to express the average response of patients with pernicious anemia adequately treated with parenteral liver extract. The maxima of the four cases of sprue treated with PGA parenterally do not differ significantly from that of the patients treated with liver extract.

more chronic cases of steatorrhea. We have observed 2 patients with sprue and an accompanying vitamin K deficiency (hypoprothrombenia) which was relieved following therapy with PGA (Darby and Jones, 1946c). Data on the hypocalcemia which sometimes occurs in sprue are not available. Hernandez-Beguerie and Spies (1946) have reported striking improvement in the intestinal motility and X-ray pattern in 2 cases of sprue treated with PGA. We have observed similar changes toward normal in the X-ray pattern of the small intestine (Darby and

TABLE I

Effect of Therapy with PGA on the Mean Corpuscular Volume in Sprue and Pernicious Anemia

Sprue									Pernicious anemia		
Patient J. D.			Patient W. O.			Patient P. S.			Patient L. G.		
Days after therapy	MCV μ^3	RBC $\times 10^6$	Days after therapy	MCV μ^3	RBC $\times 10^6$	Days after therapy	MCV μ^3	RBC $\times 10^6$	Days after therapy	MCV μ^3	RBC $\times 10^6$
−10	170	1.35	−10	134	2.53	−4	120	1.50	−8	111	2.44
15	93	3.88	−6	126	2.39	−1	112	1.61	−6	131	2.21
19	98	3.41	0	138	2.25	4	107	1.40	4	136	2.06
21	Therapy discontinued		3	135	2.15	7	110	1.45	11	128	2.50
34	117	3.47	8	136	2.58	10	100	2.31	14	116	2.92
48	108	3.98	10	134	2.68	13	79	2.77	16	113	3.10
62	103	4.38	11	151	2.38	16	88	3.28	30	121	3.22
66	100	4.69	12	130	2.78	19	87	3.22	68	106	3.86
90	93	5.18	15	115	3.14		Therapy discontinued		78	101	4.36
115	96	4.68	16	115	3.23	73	108	3.40	119	98	4.37
132	90	5.00	17	108	3.52	182	89	4.44			
167	103	3.98	18	116	3.70						
216	95	4.22	26	90	4.99						
229	95	4.18	47	95	4.75						
244	103	3.42	68	95	4.99						
258	109	2.93	96	90	4.91						
275	113	2.72									
282	126	2.39	124	94	5.12						
284	124	2.25									
293	151	1.72									
295	161	1.80									
Therapy reinstituted											
3	132	2.20									
5	122	2.38									
11	118	3.04									
22	124	3.32									
44	113	3.83									
61	118	4.00									

Jones, 1946c). These changes do not occur immediately but over a period of weeks or months.

Since decreases in the serum levels of fat-soluble vitamins occur in liver disease (Adlersberg, Sobotka, and Bogatin, 1945), one might ask whether the observed effects of PGA in man may be due to some control of storage of other factors. Such a mechanism in sprue is not ruled out, but the weight of the evidence at present indicates that the above-detailed findings reflect alterations in gastrointestinal absorption. These influences of PGA on gastrointestinal absorption in man would seem to raise the question as to the possible role of absorptive changes in inducing the picture of multiple deficiencies (Daft and Sebrell, 1945) in sulfa-treated (vitamin M-deficient) rats (Mallory *et al.*, 1944).

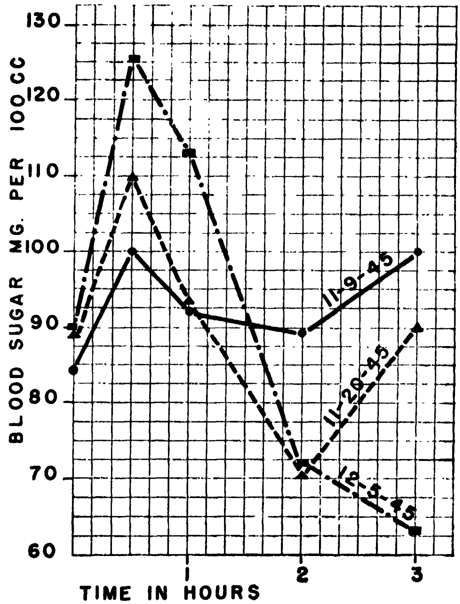


FIG. 7.—Successive oral glucose tolerance curves of a patient with sprue demonstrating the effect of therapy with PGA. Treatment instituted November 10, 1945. (Darby, Jones, and Johnson, 1946b.)

The evidence here cited may be interpreted to indicate that sprue is a disease due to a conditioned or dietary deficiency of vitamin M. PGA in small amounts appears to improve the impaired gastrointestinal absorption of glucose, fat, carotene, vitamin A, and tocopherols, the glossitis, and the anemia and leucopenia. It does not appear to be less effective than liver extract, although additional studies of the gastrointestinal effects are necessary to establish this point beyond dispute.

3. *Estimated Daily Requirements of Vitamin M*

It is well to raise the question of requirements of this vitamin. We estimated from comparative data that the daily maintenance dose would be of the order of 0.1 and 0.2 mg. while curative doses might be of the order of 1 mg. daily (Darby, Jones, and Johnson, 1946b). From study of our one case which has had a relapse after receiving a known quantity of PGA it may be estimated that the total quantity of 260 mg. of PGA sufficed to induce and maintain a remission for about 200 days (Fig. 8). This amount was given parenterally, and, hence, from the data of Heinle, Welch, and Nelson (1946) the patient may have excreted some 50% of it. Making this assumption, one may estimate therefrom a mean daily utilization of about 0.65 mg. in this case. Suárez, Spies, and Suárez (1946) reported that they had maintained good remissions in sprue on doses of 1.0 mg. daily. Hence, the requirements may be estimated to be somewhat of the order of from 0.1 to 1.0 mg.; probably about midway would be a reasonable estimate. An independent calculation by Goldsmith (1946a) based on the relapse of a case of pernicious anemia after receiving a known quantity of PGA led to an estimated requirement of

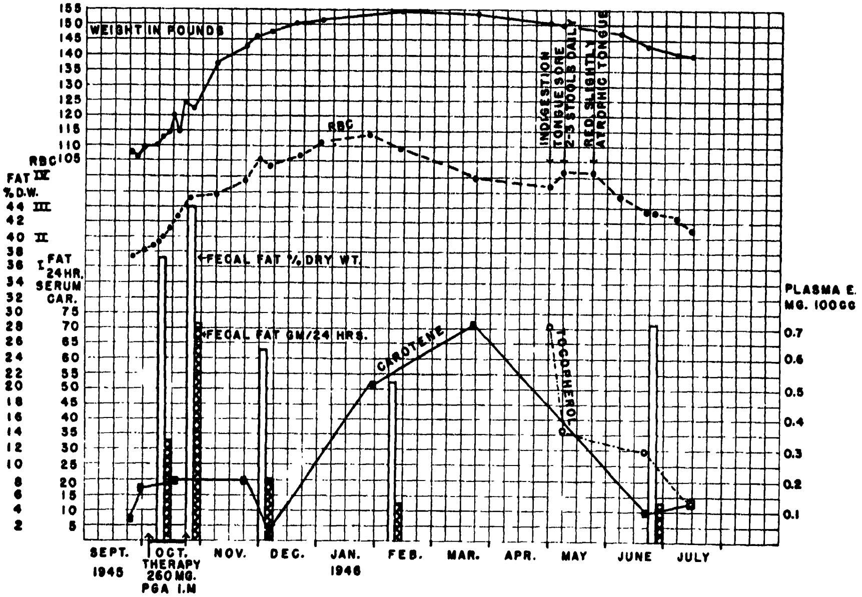


FIG. 8.—A correlation of findings in a case of sprue during a remission induced by a period of therapy with PGA and during relapse occurring after treatment was omitted. Note particularly the slow rise of carotene in the serum in remission and the general inverse relationship between serum carotene level and fecal fat.

TABLE II

The Effect of Pteroylglutamic Acid (PGA) on Serum Carotene, Vitamin A and Tocopherol Levels in Sprue[1]

Patient	Date	Carotene	Vitamin A	Tocopherol	Remarks
		γ/100 ml.	I.U./100 ml.	Mg./100 ml.	
J. D.......	9-25-45	8	98	....	Before therapy
	9-29-45	18	95	....	Before therapy
	10-4-45	...	...	....	Therapy instituted, 15 mg. PGA daily, parenterally
	10-19-45	21	129	....	Remission
	10-22-45	...	...	....	Therapy discontinued
	11-21-45	22	133	....	Remission
	12-6-45	0	137		
	1-31-46	52	153	....	Remission
	3-20-46	72	158	....	Remission
	5-1-46	...	...	0.76	Remission
	6-19-46	10	90	0.30	In relapse
	7-13-46	14	...	0.14	In relapse
	7-16-46	...	...	0.12	In relapse
	7-23-46	17	66		
	7-25-46	...	...	....	Therapy instituted, 5 mg. PGA daily by mouth
	8-26-46	54	170	....	In remission
	9-4-46	...	...	0.34	In remission
	9-25-46	56	140	0.36	In remission
P. S.......	10-18-45	12	90	....	Before therapy
	10-19-45	...	...	....	Therapy instituted, 15 mg. PGA daily, parenterally
	10-22-45	19	60		
	11-8-45	...	...	....	Therapy discontinued
	1-3-46	63	66	....	Remission
P. B.......	11-9-45	21	119	....	Chronic sprue, before therapy
	11-10-45	...	...	....	Therapy instituted, 15 mg. PGA daily, parenterally
	11-21-45	25	101	....	Remission
	12-6-45	13	89	....	Remission
	6-5-46	...	...	0.32	
	7-16-46	...	...	0.36	
	7-17-46	65	124	....	Remission
	9-25-46	79	124	0.31	Remission
W. O......	4-27-46	...	...	0.25	In relapse
	5-6-46	...	...	....	Therapy instituted, 5 mg. daily orally
	5-16-46	36	133	0.43	Early remission
	5-25-46	73	103	....	Remission
	7-13-46	51	95	0.43	Remission
	10-5-46	102	128	....	Remission

[1] Patients in Vanderbilt University Hospital (Darby, Kaser, and Jones, 1947, and unpublished observations)

similar magnitude. Day and Totter (1946) have estimated the requirement of the young monkey as between 50 and 100 γ/day when consuming 325 calories. They then postulated that the human requirement is the same as that of the monkey on an energy basis and arrived at an approximate requirement of between 300 and 600 γ for the human adult. It is encouraging that these independent lines of reasoning have led to similar estimates of requirements. They are advanced only as tentative approximations of minimal dietary requirements, however, until better and more extensive evidence can be accumulated. Whether requirements are less in the individual who does not have sprue or pernicious anemia is worthy of considering.

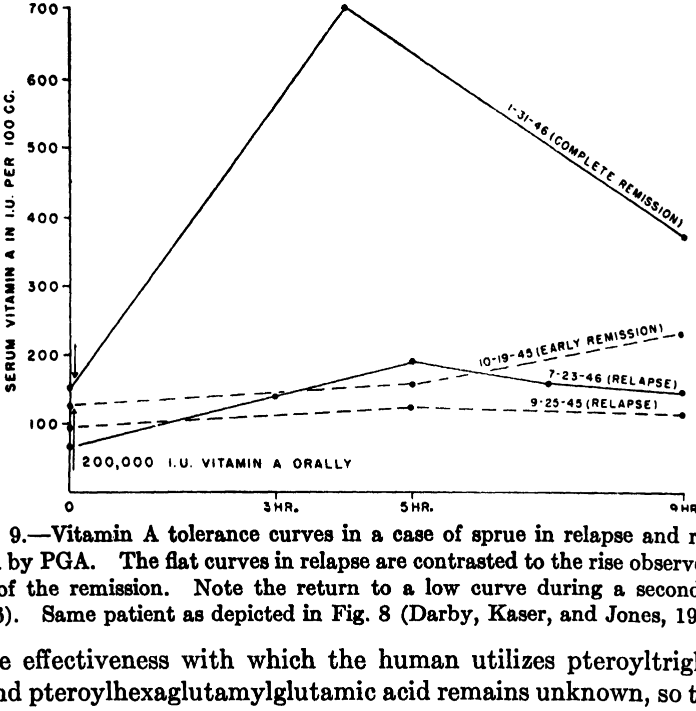


Fig. 9.—Vitamin A tolerance curves in a case of sprue in relapse and remission induced by PGA. The flat curves in relapse are contrasted to the rise observed at the height of the remission. Note the return to a low curve during a second relapse (7-23-46). Same patient as depicted in Fig. 8 (Darby, Kaser, and Jones, 1947).

The effectiveness with which the human utilizes pteroyltriglutamic acid and pteroylhexaglutamylglutamic acid remains unknown, so that the total requirement of the vitamin M group cannot yet be discussed. Likewise, reliable estimates of the vitamin M content of human dietaries cannot be made until we know the activity for the human of these several members of the complex. The evidence that the fermentation factor is utilized by man is reasonably good (Goldsmith, 1945; Spies, 1946b; Suárez, Welch *et al.*, 1946; Jones, Warden, and Darby, 1947) and there is increasing evidence that the human utilizes the heptaglutamate (Suárez, Welch *et al.*, 1946; Spies and Stone, 1947).

The possible nutritional importance of vitamin M is indicated by a report of Youmans and Patton (1946) that some 5% of a rural population in Tennessee could be classified as presenting a mild macrocytic anemia in 1939–1940. The anemic subjects were reexamined in 1946 and one-half of them (33) were found to have macrocytosis and anemia. Five of these were given 20 mg. of PGA intramuscularly and all responded with a slight but definite reticulocytosis. Oral lesions were about twice as frequent among the group with these findings as among the population generally. In this connection the reviewer has observed a case of recurrent glossitis with achlorhydria, unaccompanied by anemia or diarrhea, and which had failed to respond to all of the usual synthetic B vitamins or iron. The symptoms were promptly and completely relieved by PGA, 5 mg. every other day, and have remained so for 12 months. Whether this is an example of a very mild pernicious anemia or sprue cannot be answered. It is cited merely to indicate the need for further investigations of the effect of PGA on oral lesions.

III. NUTRITIONAL MACROCYTIC ANEMIA[3]

Nutritional macrocytic anemia is a poorly defined term applied to a group of patients with macrocytic anemia, and with the peripheral blood and bone marrow pictures indistinguishable from sprue or pernicious anemia. The cases usually exhibit free acid on gastric analysis, glossitis may or may not be present, diarrhea is inconstant, and absorptive defects have sometimes been postulated. Severe neurological involvement is uncommon. The syndrome is most frequently seen in malnourished persons and in pregnancy. The dietary history often reveals a low intake of lean meat and other animal products. It is frequently postulated that the disease is due to a dietary lack of Castle's extrinsic factor. The close similarity to sprue is evident and is further indicated by the similar frequency of occurrence in certain groups of persons and geographic areas. It is probable that some clinicians would classify certain cases as one of these two diseases while other clinicians would classify the same cases as the other. Moore *et al.* (1944) have emphasized the presence of absorptive defects in nutritional macrocytic anemia, but extensive studies are not available. The one case which we have classified as nutritional macrocytic anemia and studied extensively (Darby and Jones, 1946c) exhibited a flat glucose tolerance curve, a "deficiency pattern" upon X-ray examination of the gastrointestinal tract, somewhat low serum carotene levels and a moderately flat vitamin A tolerance. She had no definite steatorrhea on a diet of usual fat content. Free gastric acid was present after an alcohol test meal and the presence of Castle's intrinsic factor was demonstrated by the response of a case of pernicious anemia to a preparation of beef incubated with gastric juice from the patient. If this picture is general, it may be that nutritional macrocytic anemia and sprue are but different stages of the same disease. At any rate, the reported experiences by Vilter, Spies, and Koch (1945), Goldsmith (1946b; 1946d), and Heinle and Welch (1946), indicate that the hematologic and clinical responses to PGA of patients with nutritional macrocytic anemia are satisfactory. The hematologic data are insufficient to permit quantitative evaluation. They indicate, however, that the maximum reticulocytosis after therapy approximates that observed in sprue and the rate of red cell regeneration is similar to that seen in patients with pernicious anemia adequately treated with liver extract. Despite the short periods of observation, all of the twelve patients (Goldsmith, 1946b; Spies, 1946a; Heinle and Welch, 1946; Darby and Jones, 1946c) upon whom data are available to the reviewer attained erythrocyte levels greater than 3.5 million/mm.^{3} and six have exceeded 4.0 million.

---

[3] Nutritional macrocytic anemia and macrocytic anemia of pregnancy have been discussed by numerous investigators. The reader is referred to the following for clinical discussions and references: Wills (1934), Castle and Minot (1936), Wills and Evans (1938), Wintrobe (1946), Vilter (1943), Moore *et al.* (1944), and Watson and Castle (1946).

Goldsmith (1945; 1946d) observed a reticulocyte response of 12.0% on the ninth day of treatment with 5 mg. daily *per os* of fermentation *L. casei* factor of a patient with nutritional macrocytic anemia and an initial red cell count of 1.7 million. On the fourteenth day the patient had 2.5 million erythrocytes. If this observation is confirmed, it appears that there has been established another point of similarity of vitamin M deficiency in the monkey to nutritional macrocytic anemia in man, *i.e.*, both syndromes respond to either PGA or fermentation *L. casei* factor. Since nutritional macrocytic anemia in man is sometimes observed accompanying pellagra (Moore *et al.*, 1944), it is pertinent at this point to recall that the first manifestations of deficiency which monkeys exhibit on a Goldberger blacktongue-producing diet are those of vitamin M deficiency (Day *et al.*, 1940) and that a "folic acid" concentrate (Krehl and Elvehjem, 1945) or synthetic PGA (Krehl *et al.*, 1946) improved the repeated responses to niacin of dogs on a niacin-low diet (Krehl and Elvehjem, 1945).

IV. MACROCYTIC ANEMIA OF PREGNANCY[3] (PERNICIOUS ANEMIA OF PREGNANCY)

The relationship of this syndrome to sprue and nutritional macrocytic anemia is not clearly defined. It is distinguished from true pernicious anemia by virtue of its temporary character, *i.e.*, once the assumed increased demands of pregnancy have been removed by delivery, recovery may occur. Continuous therapy is not required to prevent recurrence as in the case of pernicious anemia. Gastric hydrochloric acid may be present or absent. Data on the gastrointestinal absorption in this syndrome are meager.

The first case of macrocytic anemia of pregnancy which was reported as responding to PGA was published by Moore *et al.* (1945) in December. This case and the three subsequently listed by Spies (1946a) all appear to have exhibited satisfactory reticulocyte peaks and erythrocyte regeneration. The details of additional cases of this syndrome will be awaited with interest, particularly in relation to the studies of Wills (1934) and of Watson and Castle (1946) and the clarification of the identity of the hemopoietic factor of Wills.

V. PERNICIOUS ANEMIA[4]

1. *Clinical Description*

Pernicious anemia is often referred to as Addison's anemia as a result of the clinical description of the disease by Thomas Addison in 1849 and 1855 (Kelly, 1937). It is a disease characterized by a macrocytic anemia with hyperplastic megaloblastic bone marrow, a histamine refractory achlorhydria, glossitis, and, frequently, neurological changes varying from peripheral neuritis to degenerative lesions in the spinal cord. Although pernicious anemia resembles sprue in many manifestations it is important to recognize certain differences in the two syndromes:

SPRUE	PERNICIOUS ANEMIA
Pronounced defects of absorption.	No pronounced defects of absorption (except, possibly, of tyrosine).
Gastric acid usually present.	No free gastric acid.
Often associated with poor dietary.	Not usually related to quality of diet.
Usually held to occur as celiac disease in infants.	Excessively rare before puberty.
Intrinsic gastric factor of Castle may or may not be present.	Intrinsic factor of Castle absent from gastric contents.
Pigmentation of skin.	Depigmentation of hair.

Pernicious anemia seems to be due to a metabolic defect (the absence of Castle's "intrinsic factor") which results in part in the same deficiency manifestations which one sees in sprue (defective maturation of formed elements of the blood and glossitis). The absence of the profound disturbances of gastrointestinal absorption in pernicious anemia leads one to suggest that the metabolic defect does not greatly interfere with one of the functions of PGA, *i.e.*, the maintenance of normal gastrointestinal permeability and tone. There does occur in pernicious anemia atrophy of the gastric mucosa, however. Administration of liver or liver extracts induces remissions in pernicious anemia, but their administration must be continued since the treatment is merely replacement therapy.

---

[4] Of the many exhaustive studies and reviews of pernicious anemia the reader may be referred to the following readily accessible sources for discussions of the clinical syndrome and references: Castle and Minot (1936), Wintrobe (1946), Minot and Strauss (1943), and SubbaRow, Hastings, and Elkin (1945).

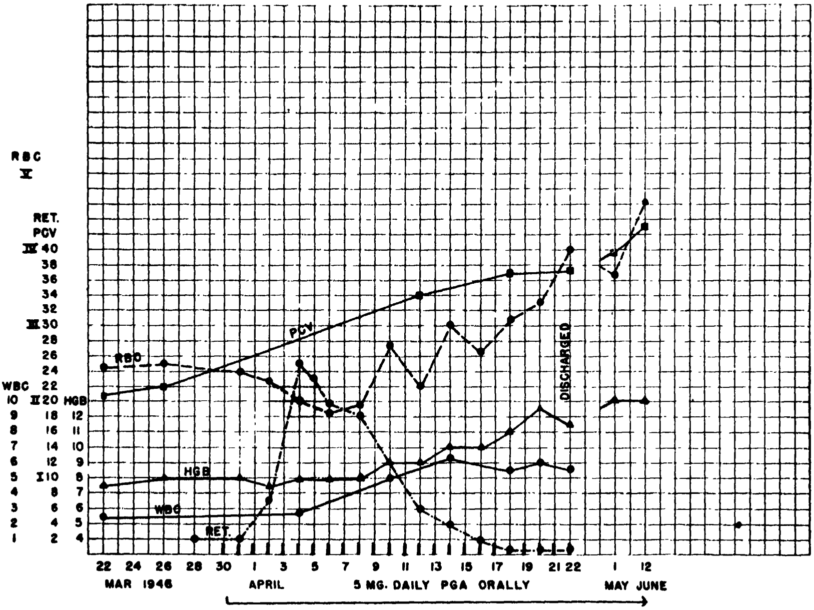


FIG. 10.—Hematologic response of a patient with pernicious anemia treated in the Vanderbilt University Hospital with PGA, 5 mg. daily by mouth. It is of interest that this patient was one of the less frequently encountered cases of pernicious anemia without macrocytosis. Note the unusually high reticulocyte peak and the attainment of satisfactory "normal" erythrocyte level.

2. *Effects of Pteroylglutamates*

a. General Clinical. Hematological responses of patients with pernicious anemia to synthetic PGA were reported during December, 1945, by both Vilter *et al.* (1945) and by Moore *et al.* (1945). The type of response reported was that expected from liver therapy (see Minot and Strauss, 1943)—improved sense of well-being, relief of glossitis, and prompt characteristic hematologic remission. This type of response of the blood and its identity with that produced by liver extract is illustrated by the cases depicted in Figs. 10 and 11. These are patients who were observed in Vanderbilt University Hospital (Jones and Darby, 1946) and were treated with 5 mg. daily of PGA by mouth. It has been demonstrated that this synthetic vitamin is effective when administered either orally (Moore *et al.*, 1945; Vilter, Spies, and Koch, 1945; Kaufmann and Schwager, 1946; Wilkinson, Israëls, and Fletcher, 1946) or parenterally (Vilter, Spies, and Koch, 1945; Doan, Wilson, and Wright, 1946; Goldsmith, 1946c; Moore *et al.*, 1946). The vitamin has also been demonstrated to be effective when administered by retention enema in quantities of 50 and 100 mg. daily (Moore *et al.*, 1946). This observation may be of considerable importance in view of the possible synthesis of the substance by the gastrointestinal flora in the human.

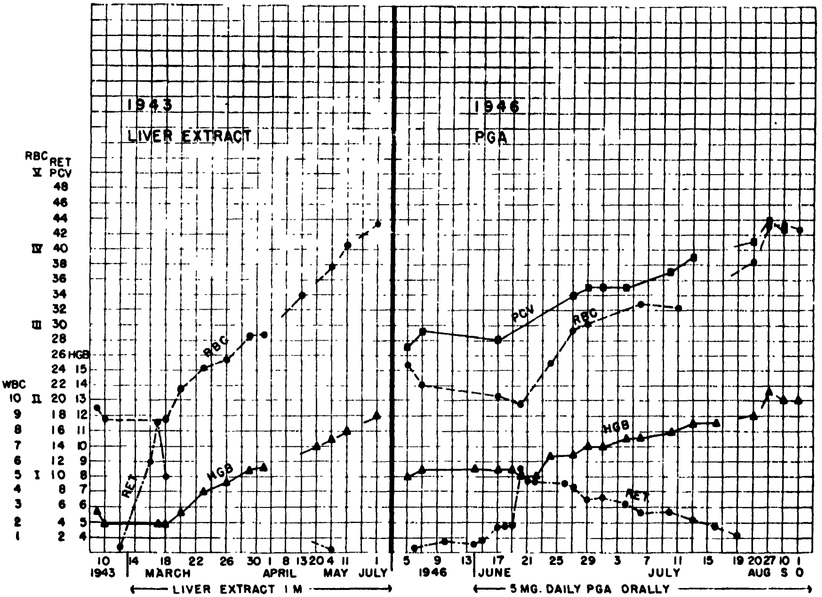


FIG. 11.—Hematologic responses of a patient with pernicious anemia treated in the Vanderbilt University Hospital, first in 1943 with parenterally administered liver extract. Received a total of 130 units from March 13 to July 1, 1943. Discontinued liver extract at latter date and received no medication until seen in the subsequent relapse in June, 1946. Then received 5 mg. of PGA orally beginning June 14 as indicated. The identical character of the two remissions is evident.

Moore *et al.* (1945) critically considered the question of the relationship of PGA to the anti-pernicious anemia principle. They pointed out that since it was effective when administered orally without gastric juice and when given intravenously, that it did not correspond to the extrinsic factor of Castle. Furthermore, since potent liver extracts containing as little as 0.035 mg. of solids have been prepared and since various liver extracts which are active in pernicious anemia often contain almost no *L. casei* factor activity, they concluded that this vitamin could not be responsible for all of the activity of liver extracts. It is to be recalled, also, that certain liver extracts with anti-pernicious anemia potency had been shown to be ineffective in the anemia of monkeys and chicks in the doses tested.

Prolonged remissions have been induced with PGA in a sufficient number of patients with pernicious anemia that it is certain the substance can effect erythrocyte maturation in this disease. It is of considerable interest, therefore, to compare the responses of patients so treated with the responses of patients with pernicious anemia who have been treated with liver extracts. Fortunately, there exists a body of standards for the response of patients adequately treated with liver extracts. All of the data available to the reviewer have been compared with certain of these standards (Figs. 12, 13, 14 and 15). Because of the paucity of data on patients treated continuously for even as long as three months, these analyses have been limited to the early part of the remissions.

b. Reticulocyte Response. The reticulocytosis which is characteristic of hematologic remission in pernicious anemia reached a maximum between the third and eleventh day (mean, 7.5 days) in 36 cases following therapy with 5 mg. or more of PGA (data of Castle and Bauer, 1946; Goldsmith, 1946b; Heinle and Welch, 1946; Jones and Darby, 1946; Kaufmann and Schwager, 1946; Moore *et al.*, 1945; Shukers, 1946; Vilter, Spies, and Koch, 1945). There was no significant difference in the time of occurrence of the maximum between those patients who received the vitamin orally and those getting it parenterally. The height of the reticulocyte peak in pernicious anemia is dependent upon several factors (Minot and Castle, 1935): (1) the quantity of active substance administered; (2) the route of administration; (3) the pretreatment level of the erythrocytes; and (4) the reactive state of the bone marrow. In the analyses provided, an effort has been made to compare the responses to liver extract and PGA under comparable conditions of administration and with allowances for the pretreatment level of erythrocytes. Comparisons of this sort are valid only when they consist of groups of patients. It cannot be overemphasized that the so-called standards are merely curves or lines which most nearly express the means of a whole series of observations. Obviously, many responses will fall above and many below the "expected" or standard curves so derived. Investigators have not always recognized this fact in the effort to generalize conclusions regarding dosage level or activity from a single case or a very few cases. It is probable that many of the discrepancies in the chemical fractionation of liver (SubbaRow, Hastings, and Elkin, 1945) have arisen from interpretation of differences between individual patients rather than true differences in the activities of fractions tested.

The reticulocyte maxima of 26 cases of pernicious anemia who were treated with PGA orally in eight different clinics are depicted in Fig. 12. The data are limited to patients obtaining 5 mg. or more of PGA daily. For comparison the standard reticulocyte response derived by Riddle (1930) for patients adequately treated with orally administered liver is shown. Two deductions are possible from these data: (1) The reticulocyte maxima of these patients treated with PGA is at least as high, if not higher, than the standard curve for orally administered liver. (2) The data are consistent from the various clinics.

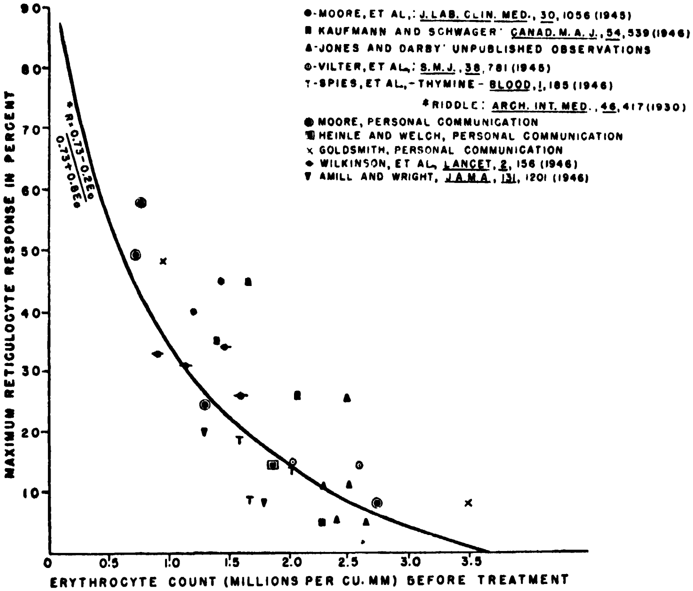


FIG. 12.—Comparison of maximum reticulocyte responses of patients with pernicious anemia treated with orally administered liver extract with patients treated with PGA or thymine orally. The curve is that of the indicated formula as derived by Riddle (1930) to express the average response of patients adequately treated with liver extract. Note that more of the maxima of patients treated with PGA orally fell above this standard than below it.

A similar comparison of the reticulocyte maxima reported from eight clinics for 24 cases treated with PGA parenterally (most cases were treated intramuscularly) is made in Fig. 13. The standard for comparison is the mean response of patients adequately treated with intramuscular liver extract (Isaacs and Friedman, 1938). The dosages were grouped as indicated. A study of the data revealed that they were comparable from clinic to clinic. From the figure it is readily apparent that the reticulocyte maxima of these cases treated parenterally failed to cluster about the standard or "expected" response. Furthermore, it is not possible to be certain that there exists a real difference in the response to the lowest dosages employed (0.5–1.0 mg.) and the response to 5–20 mg. daily. Moore (1946) observed no secondary response to liver extract administered to two patients following periods of therapy with 1 mg. daily of PGA intramuscularly. Some patients, therefore, respond maximally to as little as 1.0 mg. of PGA. Finally, for comparison of the effectiveness of the two methods of administration of the vitamin the data from Fig. 12 have been superimposed on Fig. 13. There seems to be a trend for the patients who received PGA orally to exhibit higher reticulocyte maxima than those receiving it parenterally. Several possi-

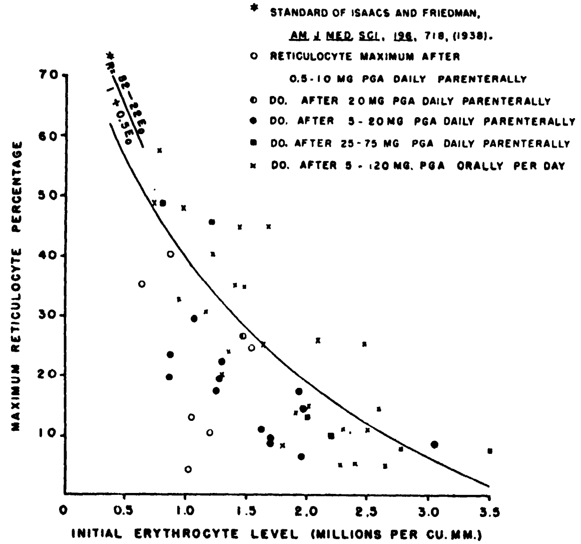


FIG. 13.—Comparison of reticulocyte maxima in pernicious anemia treated with PGA parenterally or orally with standards for intramuscular liver. The curve is that of the indicated formula as derived by Isaacs and Friedman (1938) to express the average response of patients adequately treated with parenteral liver extract. The circles indicate the reticulocyte maxima of patients treated with PGA parenterally, the crosses the maxima of patients treated with PGA orally. Note that at least 4 of the patients receiving 2 mg. or less of PGA daily responded equally as well as those receiving several times as much. Also, note that the patients who received oral therapy exhibited higher reticulocyte maxima than those treated parenterally. (Data of Castle and Bauer, 1946; Doan, Wilson, and Wright, 1946; Goldsmith, 1946b; Heinle and Welch, 1946; Jones and Darby, 1946; Kaufmann and Schwager, 1946; Moore *et al.*, 1945; Moore, 1946; Vilter, Spies, and Koch, 1945; Wilkinson, Israëls, and Fletcher, 1946; Amill and Wright, 1946.)

ble explanations of this observation occur: (1) The parenterally administered material is more rapidly excreted (Heinle, Welch, and Nelson, 1946) and, therefore, it may be less completely utilized. (2) The oral dosages may have been larger. This latter seems an unlikely explanation as there was no difference between the reticulocyte response to 5 mg. and 100 mg. administered orally, and, as noted above, the parenteral dosage range studied did not determine the height of the response. (3) The activity of PGA may actually be enhanced by or during absorption from the gastrointestinal tract. It is impossible to choose between these explanations. Perhaps the first one has more evidence in its favor.

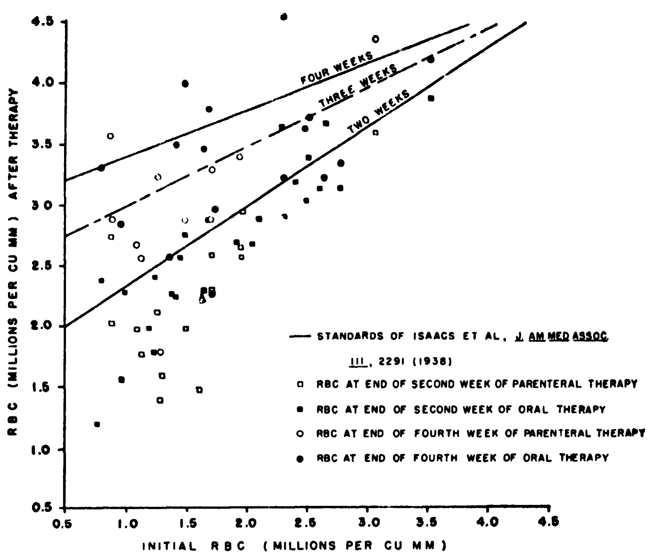


FIG. 14.—Comparison of increases in RBC of patients with pernicious anemia treated with PGA with standards for RBC increases following intramuscular liver extract. According to the standards depicted by the lines, the average erythrocyte level of patients adequately treated with liver extract parenterally, whose initial red cell count was 2.0 million, would be 3.0 million after two weeks and 3.78 million at 4 weeks. Regeneration appears to be slower after administration of PGA than these standards, but, possibly, more nearly approaches the standards following oral therapy. Sources of the data are the same as indicated in Fig. 13. Analysis limited to patients receiving 5 mg. or more of PGA daily.

c. Erythrocyte Response. Since the reticulocyte response alone is not so dependable a measure of the activity of a hemapoietic substance as is the rate of regeneration of erythrocytes (Minot and Strauss, 1943), it is of interest to examine this rate. The dearth of data has restricted the analysis to the first four weeks of therapy. A comparison of the increases in erythrocytes of patients two and four weeks after therapy with PGA was instituted is made in Fig. 14. The data for both routes of administration have been included, the dosage level restricted to patients receiving 5 mg. daily. Considerable variation was present in the responses to either method of administration. Again the data from all nine clinics represented appeared comparable. The erythrocyte level at both two and four weeks after therapy with PGA appeared to be slightly below the standards proposed by Isaacs *et al.* (1938) for patients adequately treated with liver extract parenterally. There appears little difference in the levels of patients treated by the two routes; if any difference is apparent it is again in favor of the oral route. Another desirable comparison is the erythrocyte response of patients treated with PGA orally to that of patients treated with desiccated stomach tissue orally. Fig. 15 illustrates this. The two responses are very similar.

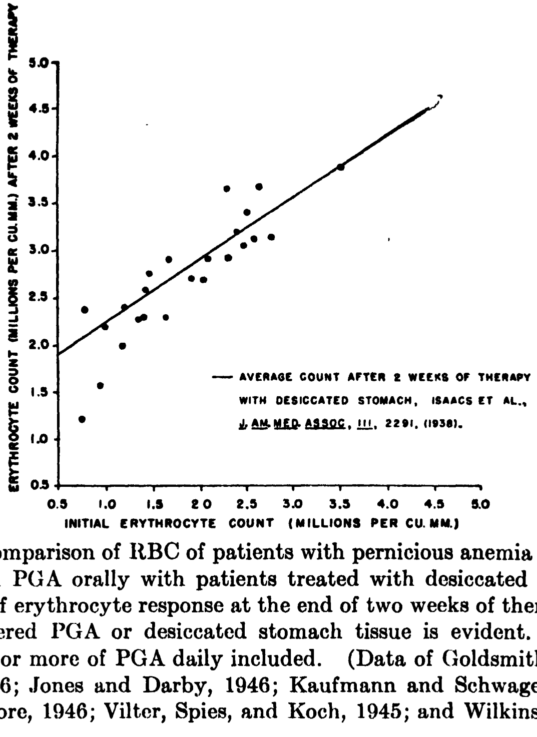


FIG. 15.—Comparison of RBC of patients with pernicious anemia after two weeks of therapy with PGA orally with patients treated with desiccated stomach tissue. The similarity of erythrocyte response at the end of two weeks of therapy with either orally administered PGA or desiccated stomach tissue is evident. Only patients receiving 5 mg. or more of PGA daily included. (Data of Goldsmith 1946b; Heinle and Welch, 1946; Jones and Darby, 1946; Kaufmann and Schwager, 1946; Moore *et al.*, 1945; Moore, 1946; Vilter, Spies, and Koch, 1945; and Wilkinson, Israëls, and Fletcher, 1946.)

One may ask whether the erythrocyte level can be raised to its maximum by administration of PGA alone or whether some other factor is required. The data depicted in Figs. 10 and 11 illustrate that, in some cases at least, this vitamin alone suffices to increase the red cell count to obviously normal limits. Although 5 million erythrocytes/mm.^{3} is generally an arbitrary standard for men and 4.5 million for women, it is not to be expected that all patients can be raised to these levels by any form of therapy. Of the 12 cases (Castle and Bauer, 1946; Moore, 1946; Kaufmann and Schwager, 1946; Jones and Darby, 1946) who have received PGA alone continuously for eight weeks (56 days) or more the mean erythrocyte count was 4.37 million and all but one of the patients had attained stable levels above 4.0 million. Nine of these cases have maintained satisfactory blood levels on therapy with PGA only for periods greater than four months (16 weeks). It is the reviewer's present impression that satisfactory erythrocyte levels can be attained in most cases of pernicious anemia by treatment with PGA only and that the failure to observe such levels has sometimes been due to the impatience of the investigator. In our experience the red cell count often continues to rise gradually for a period of some three months after therapy is initiated. This may occur even if therapy is interrupted after a period of intensive treatment. Hence, conservatism must be enjoined in interpreting the effectiveness of an agent which is instituted subsequent to a period of less than three or four months of treatment with PGA.

The reduction of the macrocytosis under therapy which has been observed is illustrated in Table I. Four cases treated with PGA intramuscularly (Castle and Bauer, 1946) have shown reduction in the mean corpuscular volume to the range of 88–101 μ^3 and five patients treated with PGA orally (Jones and Darby, 1946) have shown decreases in the MCV to the range of 92–105. The hemoglobin increases compare favorably with the changes in erythrocytes. Where good responses in red cells have been observed there has also appeared a moderate rise in the leucocytes. Likewise, there has been noted an outpouring of platelets following therapy with PGA. The increase in platelets is detectable just after the reticulocytosis begins and it appears that the platelets remain elevated for several days (Moore *et al.*, 1945; Jones and Darby, 1946). This same effect occurs in sprue (Moore *et al.*, 1945; Darby, Jones, and Johnson, 1946b). We have observed one case of nutritional macrocytic anemia in which the platelets attained the astonishing level of 5.4 million/mm.^{3} 13 days after treatment with intramuscular PGA was instituted.

d. Other Effects. It is impossible yet to evaluate the effectiveness of PGA in treating the neurological aspects of pernicious anemia. Two investigators have reported that the mild symptoms of paresthesias have improved following therapy with PGA (Doan, Wilson, and Wright, 1946; Kaufmann and Schwager, 1946). No published report of the improvement of definite combined system disease has yet appeared. Moore (1946) has observed a patient who exhibited a satisfactory hematologic response to PGA but who developed a cellulitis and, despite continuous daily oral doses of 100 mg. of PGA, this patient showed "marked increase in symptoms and signs of combined system disease." Recent reports indicate that PGA is ineffective in preventing the extension of neurologic damage in some cases of pernicious anemia (Heinle and Welch, 1947; Vilter, Vilter, and Spies, 1947).

Three rather striking cases indicate that the effectiveness of PGA in pernicious anemia may not always be complete. Heinle and Welch (1946) have observed a patient with pernicious anemia who showed a rather poor response to the synthetic factor in doses up to 12 mg. daily over a period of six weeks but who then exhibited a prompt secondary response to intramuscular liver extract. Shukers (1946) reports two patients with pernicious anemia in whom PGA was not effective; both of his patients had neurological complications. One of them initially exhibited a definite hematological betterment but later, while taking 10–15 mg. of PGA daily, underwent a clinical and hematologic relapse. Parenterally administered liver extract promptly restored the erythrocyte count to higher levels than previously attained with PGA. The clarification of such experiences awaits further study. Spies (1946a) also states that he has observed a number of patients with "macrocytic anemia in relapse" treated with 3 or 4 mg. of "folic acid" who have later shown secondary responses to liver extract or yeast in quantities supplying 1 mg. or less of "folic acid."

3. *Evidence Relative to the Nature of a Metabolic Defect*

The developments in the study of pteroylglutamates may clarify our concept of the pathogenesis of pernicious anemia. Much of the following discussion of these developments is based largely on preliminary data and cannot be critically evaluated at the time of writing. It is presented as background for an attractive tentative hypothesis.

Wintrobe (1939) demonstrated that very large doses of yeast, which contains primarily "potential folic acid" (Mims, Totter, and Day, 1944), could induce a remission in patients with pernicious anemia. Sharp and Vonder Heide (1944) observed that remissions did not occur in 6 cases of relapsed pernicious anemia who received 400–1500 γ of "concentrates of vitamin B_c made from yeast or liver." Castle *et al.* (1944) likewise found no response in two cases whom they treated with fermentation *L. casei* factor. Spies (1946b) has recently reported a questionable response of a case of pernicious anemia to the daily injections of 3 mg. of the latter factor.

It was reasonable to conclude that the more complex pteroylglutamates are poorly utilized, if at all, by the patient with pernicious anemia.

Bethell *et al.* (1946) have reported that a yeast concentrate of B_c conjugate failed to induce a response in two patients with pernicious anemia and one patient with a macrocytic anemia following gastrectomy. These patients were given the equivalent of 2.3, 4.0 and 4.0 mg. of PGA as the conjugate. Subsequently an equivalent quantity of PGA was administered to one of the patients with pernicious anemia and the gastrectomized subject. A submaximal response followed. The second case of pernicious anemia was treated with the same preparation of B_c conjugate after it had been subjected to the enzyme which deconjugates the heptaglutamate. A response occurred. These workers also report that the oral administration of PGA to either healthy subjects or individuals with pernicious anemia results in the augmentation of the urinary excretion of the material. The administration of vitamin B_c conjugate, however, resulted in the increased excretion of the free vitamin (PGA) only in the normal subject. They suggested that in pernicious anemia "the conjugated forms of folic acid of the diet are not efficiently converted to the free state."

Similar results were obtained in a healthy subject and in two patients with pernicious anemia who were studied by Welch *et al.* (1946). These workers extended their findings to demonstrate (1) the absence from normal gastric juice of an enzyme which liberates PGA from the heptaglutamate; (2) that the addition of purified liver extract to various dilutions of bone marrow extracts from healthy subjects and patients with pernicious anemia "augmented frequently (50–150%) the formation of *L. casei* factor from added heptaglutamate, *in vitro* at pH 4.5, but not at pH 7.0," and (3) that injections of purified liver extracts (which contained a negligible quantity of free *L. casei* factor) increased the urinary excretion of *L. casei* factor by two subjects with pernicious anemia.[5]

A tentative hypothesis[6] was advanced that in pernicious anemia there is a metabolic defect which results in the decrease of or inactivation of a tissue enzyme similar to that described by Mims, Totter, and Day (1944) and Binkley *et al.* (1944), termed vitamin B_c conjugase (Bird *et al.*, 1945) and now characterized as a carboxypolypeptidase (Pfiffner *et al.*, 1946). The supplying of free *L. casei* factor (PGA) may be considered to by-pass the metabolic defect which has resulted in the inability to utilize efficiently the total vitamin M group in the diet. As attractive as this hypothesis is, it does not identify the intrinsic factor of Castle with conjugase because the latter is stated to be absent from normal gastric juice. A corollary of this hypothesis might be that pernicious anemia, sprue, tropical macrocytic anemia of Wills, nutritional macrocytic anemia as observed in this country, and the macrocytic anemia of pregnancy, all have in common a deficiency of pteroylglutamic acid or some metabolite of it. The deficiency in pernicious anemia would be due to a metabolic defect which prevented the body from obtaining the free PGA from the foodstuffs. In tropical and nutritional macrocytic anemia and sprue the deficiency might be considered as dietary or conditioned in origin. One fact strongly in favor of this unitarian hypothesis is that these syndromes are similar clinically and that they have in common the megaloblastic arrest of the marrow. Although the accompanying parts of the syndromes might be reconcilable with this unitarian hypothesis, it is difficult to explain the activity of purified liver extracts in the nutritional groups on this basis. Alternative hypotheses might be that liver extract contains a metabolite of PGA which is active in all of the reactions in which the two substances participate in common, or that the inactivation of an antimetabolite is involved. Whatever the ultimate clarification of the problem, it is apparent that the availability of the pure vitamin M compounds will serve as useful tools for study and, perhaps, elucidation of the defect. They may also serve to reclassify clinically similar syndromes.

---

[5] In evaluating the reported data which are interpreted to indicate that liver extract augments the urinary excretion of PGA, it is well to recall that vitamin M is a water-soluble factor. The increased excretions of PGA which have been observed after dosing with small amounts of conjugated forms may be related to the phenomenon of saturation rather than to the administered liver extract.

[6] Evidence which has appeared since this discussion was written makes necessary a revision of the simple hypothesis but does not permit formulation of a substitute one.

If confirmed, this evidence for the existence in pernicious anemia of a defect in the metabolism of a vitamin resulting in a picture of a deficiency may usher in a new era in our studies of the metabolic diseases. Perhaps other metabolic diseases which resemble experimental deficiencies are due to metabolic defects at such levels that they cannot be corrected by administering the vitamin alone. For example, pseudohypertrophic muscular dystrophy cannot be cured by giving vitamin E despite the many similarities of experimental vitamin E deficiency and the dystrophy in man (Minot and Frank, 1944). Minot and Frank, among others, have postulated that "It is conceivable that some interference with . . . intermediate steps blocks the effectiveness of tocopherol in the muscles of patients with clinical dystrophy and so brings about essentially the same metabolic failure as occurs in animals deprived of vitamin E." Such a concept is analogous to the present-day speculation regarding the above-reviewed anemias.

VI. PTEROYLGLUTAMIC ACID IN TREATMENT OF DISEASES OF INFANCY AND CHILDHOOD

There are at least four conditions of interest here which occur in infancy and childhood. The first of these is celiac disease—a steatorrhea which resembles sprue in all of its manifestations except that anemia is less common. It is often considered to be infantile sprue and liver extract is employed in the treatment of the syndrome. Only one published case report of the influence of PGA on celiac disease has appeared. Goldsmith (1946b) has observed one case of a celiac syndrome which appeared to improve following therapy with PGA.

The second pediatric condition of interest is extremely rare—true pernicious anemia of infancy and childhood. Peterson and Dunn (1946), of Vanderbilt University, have critically reviewed the reports of this disease and have found but two such cases which met their criteria. These criteria are:

1. Macrocytic anemia.
2. Histamine-resistant achlorhydria.
3. Hyperplastic bone marrow with megaloblastic arrest.
4. Specific response (reticulocytosis) following liver therapy.
5. Necessity of continuous therapy to maintain a continuous remission.

They have added a third patient who has been observed through 5 relapses between the age of 13 months and 5½ years. Four remissions were induced by liver extract and a fifth by orally administered PGA (Peterson, 1946). This response was identical in every way with those remissions following liver extract.

Finally, there is an anemia which is not infrequent in infancy and which one might characterize as a temporary pernicious anemia. Cases of this disease have been studied in many localities. The hematologic picture is that of pernicious anemia from which the syndrome differs merely in that no relapse occurs following one course of therapy. The disease is often attributed to a lack of the extrinsic factor and, therefore, is frequently described as nutritional in origin. In this connection it may be noted that of 25 such cases studied by Zuelzer and Ogden (1946b), 23 of them were artificially fed and one of these had been receiving goat's milk. Fourteen of the 25 infants had received substandard diets. No chronic malabsorption from the gastrointestinal tract was observed, but infections, especially respiratory infections, were frequent.

Zuelzer and Ogden (1946b) have reported that this megaloblastic anemia of infancy responds completely to orally or intramuscularly administered synthetic PGA. The response did not differ from that obtained from liver extract. These workers conclude that "folic acid is identical with or contains a hemapoietic factor which has a specific effect on megaloblastic anemia. This effect is indistinguishable from that produced by purified commercial liver extract."

Finally, another anemia of infancy known as goat's milk anemia, a macrocytic anemia which is stated to occur in children fed exclusively on goat's milk, may be related to a deficiency of the vitamin M group. No reports have appeared on the effectiveness of PGA in this disease.

VII. PGA IN THE TREATMENT OF CHRONIC DIARRHEA

Carruthers (1946) has published a brief paper from India reporting 6 cases of incompletely classified chronic diarrhea which had been treated with PGA. Administration of PGA in oral doses of 50–60 mg. daily was followed by a return of the stools to normal within 2–5 days. He concluded that a nutritional deficiency probably explained the persistence of the diarrhea in these cases. It is of interest that his brief case reports indicate that three of these cases had an accompanying anemia of less than 2.0 million red cells and the other three had either bacillary or amebic dysentery. Shukers (1946) has seen two cases of chronic diarrhea subside while receiving PGA. These experiences call to mind the statement of McCarrison which was quoted earlier in this review, as well as an observation of Day *et al.* (1940) that: " . . . a deficiency (in the monkey) of what we term vitamin M results in a lowered resistance to infection of the mucosa of the alimentary tract. Thus, one may observe lesions of the mouth or large intestine, or of both, which appear to be brought about by organisms which would seem to have been present in those locations prior to the deficient feeding, but which were not causing pathological changes." A similar observation was later made by Waisman *et al.* (1943).

It has been suggested that dysentery is in part an infectious disease engrafted upon a nutritional deficiency of vitamin M (Editorial, 1941). No conclusion can be reached regarding the role of vitamin M in diarrheas other than sprue until a great many more studies are made. The question is raised here merely in hopes of stimulating further investigations of the subject.

VIII. THYMINE

Four reports by Spies and his co-workers (Spies *et al.*, 1946a,f; Frommeyer *et al.*, 1946; Spies *et al.*, 1946g) indicate that thymine in very large doses is effective in producing remissions in cases of sprue and pernicious anemia. The reticulocyte remissions in sprue after thymine were within the range of those observed in adequately treated patients (Fig. 5). The published data do not extend over a sufficiently long period of time to enable any final evaluation of the effectiveness of this pyrimidine in bringing about red cell regeneration. None of the 6 reported cases of pernicious anemia reached erythrocyte levels of 3.0 million. In all, these workers have recorded 6 cases of pernicious anemia and 4 cases of sprue treated with this material. They state that the clinical response of sprue to doses of 15 g. daily of thymine is less dramatic than that observed with PGA.

The dosage employed to induce these remissions has been 4.5 g. daily in pernicious anemia and 15 g. daily for sprue—1,000 to 3,000 times the dosage of PGA which has proved satisfactory for treatment of these diseases.

These studies were suggested by the generally known ability of thymine to substitute for *L. casei* factor in the growth of the lactic acid bacteria. Following the microbiologist, Spies *et al.* (1946b) have suggested that PGA may act as an enzyme or coenzyme in the synthesis of thymine or a thymine-like substance. The fate of this hypothesis and the mechanism of action of this vitamer are not decided.

IX. A RESUMÉ OF THE NUTRITIONAL PHYSIOLOGY OF VITAMIN M IN MAN

The vitamin M-deficient monkey utilizes PGA, pteroyltriglutamate, and pteroylheptaglutamate. The importance for this species of the "potential folic acid" in foodstuffs was early demonstrated by the Arkansas group (Mallory *et al.*, 1944). If one assumes that vitamin M deficiency in the monkey is the experimental analogue of the various deficiency syndromes in man, except pernicious anemia, which have been considered in this review, the importance of these conjugated forms in human nutrition becomes apparent. Evidence is accumulating that these conjugated forms are active in man. The excretion studies of Bethell *et al.* (1946) and of Welch *et al.* (1946) indicate that they may be available to the normal human. It would be logical, therefore, to standardize the expression of the vitamin M content of foodstuffs by some means such as PGA-equivalency after incubation with an enzyme which liberates this free substance. Such a method of assay was proposed by Mims, Totter, and Day (1944). The choice of a method of liberation, however, has not been simple (Luckey *et al.*, 1945), but it should be easier now that the chemistry of the factors is revealed.

The requirements of this vitamin have been discussed (page 136) and its effectiveness upon oral, parenteral, or rectal administration noted. The future studies of the relative importance of dietary sources and intestinal synthesis may well supply much needed answers to the question of the pathogenesis of sprue, nutritional macrocytic anemia, tropical macrocytic anemia, macrocytic anemias of pregnancy, and the macrocytic (megaloblastic) anemias of infancy.

The gross physiological effects of the vitamin were considered under Sprue (page 125). From the effects which the substance exerts on the maturation of the cells of the bone marrow and the rapidly growing oviduct of the chick (Hertz and Sebrell, 1944; Hertz, 1945) it is apparent that the vitamin is intimately concerned with rapid cell division and growth. The observation by Hertz (1945) that rather large quantities of the vitamin are required to induce in the chick maximum oviduct response to stilbestrol may indicate that pregnancy does, in fact, increase considerably the requirement of these factors and, thereby, account for the relative frequency of the deficiency anemias and sprue during pregnancy. No demonstration has been made of the exact role of the substance in an enzymatic reaction. The studies on thymine (page 154) and of a possible relationship to protein metabolism (Kornberg, Daft, and Sebrell, 1946) may be of importance in this regard.

It is of interest that the point of action of PGA on the marrow in all of the anemias in which it is effective is the megaloblast. This observation permits a classification of the anemias which are affected by the substance and may assist in defining the exact role of the vitamin in the tissues. Relative to the mechanism of the gastrointestinal effects, Davis (1946) has stated that PGA increases the serum content of cholinesterase. Until data are published in full, judgment of this claimed effect must be withheld.

Unlike other water-soluble factors, there appears to be, on ordinary intakes, only slight urinary excretion of *L. casei* factor or "folic acid." Proportionately larger amounts are excreted in the perspiration than occurs with the other water-soluble vitamins; the ratio of dermal excretion to urinary excretion under hot moist environmental conditions is approximately 5 or 6 (Johnson, Hamilton, and Mitchell, 1945). The dermal loss is so small that it probably does not alter the requirement materially. However, it may be that the combination of poor dietary and increased excretion of the substance via the perspiration in tropical environments accounts in part for the geographical distribution of the syndromes now recognized as vitamin M deficiencies. The excretion of "potential folic acid" in milk (Wright *et al.*, 1945) has not yet been completely clarified.

The evidence here reviewed demonstrates that the vitamin M complex is of importance in human nutrition. The incidence of dietary deficiencies of this vitamin in the general population is unknown. It is most probable that the incidence is low in the United States but higher in certain tropical areas. Until our understanding of the vitamin and its deficiency manifestations are greatly clarified, the reviewer would caution that attempts to fortify foodstuffs with the factor would be most premature and, conceivably, not without potential harm to patients with combined system disease.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to the several workers who have so generously permitted the use of their unpublished observations in the preparation of the review. In particular he wishes to acknowledge the helpful advice of his collaborator, Doctor Edgar Jones.

The previously unpublished data from Vanderbilt University Hospital were accumulated as part of the studies of the Tennessee-Vanderbilt Nutrition Project which is supported by the Nutrition Foundation, Inc., the International Health Division of The Rockefeller Foundation, the Tennessee Department of Public Health, and the National Vitamin Foundation.

REFERENCES

1. Adlersberg, D., Sobotka, H., and Bogatin, B. 1945. *Gastroenterology* **4,** 164–174.

1a. Amill, L. A., and Wright, M 1946 *J. Am. Med. Assoc.* **131,** 1201–1207.

2. Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., and Waller, C. W. 1946. *Science* **103,** 667–669.

3. Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., Waller, C. W., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1945. *Science* **102,** 227-228.

4. Anonymous. 1944. *Nutrition Revs.* **2,** 54–55, 103–105, 221–222.

5. Anonymous. 1945. *Nutrition Revs.* **3,** 165–167.

6. Anonymous. 1946. *Nutrition Revs.* **4,** 11–17, 91–93, 102–104, 163–168, 199–200.

7. Barker, W. H. 1943. *Med. Clinics N. America* **27,** 451–465.

8. Berry, L. J., and Spies, T. D. 1946. *Blood* **1,** 271–306.

9. Berry, L. J., Spies, T. D., and Doan, C. A. 1945. *Southern Med. J.* **38,** 590–592.

10. Bethell, F. H., Swendseid, M. E., Bird, O. D., Meyers, M. C., Andrews, G. A., and Brown, R. A. 1946. *Univ. Mich. Hosp. Bull.* **12,** 42–43.

11. Binkley, S. B., Bird, O. D., Bloom, E. S., Brown, R. A., Calkins, D. G., Campbell, C. J., Emmett, A. D., and Pfiffner, J. J. 1944. *Science* **100,** 36–37.

12. Bird, O. D., Binkley, S. B., Bloom, E. S., Emmett, A. D., and Pfiffner, J. J. 1945. *J. Biol. Chem.* **157,** 413–414.

13. Black, D. A. K., Fourman, P., and Trinder, P. 1946. *Brit. Med. J.* **2,** 418–420.

14. Carruthers, L. B. 1946. *Lancet* **1,** 849–850.

15. Castle, W. B., and Bauer, J. L. 1946. Personal communication.

16. Castle, W. B., and Minot, G. R. 1936. Pathological Physiology and Clinical Description of the Anemias. Oxford Univ. Press, New York.

17. Castle, W. B., Rhoads, C. P., Lawson, H. A., and Payne, G. C. 1935. *Arch. Internal Med.* **56,** 627–699.

18. Castle, W. B., Ross, J. B., Davidson, C. S., Burchenal, J. H., Fox, H. J., and Ham, T. H. 1944. *Science* **100,** 81–83.

19. Clark, G. W. 1945. *Am. J. Med. Sci.* **209,** 520–524.

20. Cooperman, J. M., Elvehjem, C. A., McCall, K. B., and Ruegamer, W. R. 1946. *Proc. Soc. Exptl. Biol. Med.* **61,** 92–97.

21. Daft, F. S. 1946. *Ann. N.Y. Acad. Sci.* **48,** 299–307.

22. Daft, F. S., and Sebrell, W. H. 1945. *Vitamins & Hormones* **3,** 49–72.

23. Darby, W. J., Cherrington, M. E., and Ruffin, J. M. 1946. *Proc. Soc. Exptl. Biol. Med.* **63,** 310–312.

24. Darby, W. J., and Jones, E. 1945a. *Proc. Soc. Exptl. Biol. Med.* **60,** 259–260.
25. Darby, W. J., and Jones, E. 1945b. Proc. Southern Section, Am. Fed. Clin. Research, Atlanta Meeting, Dec. 8.
26. Darby, W. J., and Jones, E. 1946. Unpublished observations.
27. Darby, W. J., Jones, E., and Johnson, H. C. 1946a. *Science* **103,** 108.
28. Darby, W. J., Jones, E., and Johnson, H. C. 1946b. *J. Am. Med. Assoc.* **130,** 780–786.
29. Darby, W. J., Kaser, M. M., Jones, E. 1947. *J. Nutrition* **33,** 243–250.
30. Davis, J. E. 1946. *Science* **104,** 37–38.
31. Day, P. L. 1944. *Vitamins & Hormones* **2,** 71–105.
32. Day, P. L., Langston, W. C., and Darby, W. J. 1938. *Proc. Soc. Exptl. Biol. Med.* **38,** 860–863.
33. Day, P. L., Langston, W. C., Darby, W. J., Wahlin, J. G., and Mims, V. 1940. *J. Exptl. Med.* **72,** 463–477.
34. Day, P. L., Mims, V., and Totter, J. R. 1945. *J. Biol. Chem.* **161,** 45–52.
35. Day, P. L., Mims, V., Totter, J. R., Stokstad, E. L. R., Hutchings, B. L., and Sloane, N. H. 1945. *J. Biol. Chem.* **157,** 423–424.
36. Day, P. L., and Totter, J. R. 1946. Reported at the AAAS Conference on Vitamins, Gibson Island, July 31, 1946.
37. Day, P. L., and Totter, J. R. 1947. *Biological Symposia* **12,** 313–338. Jaques Cattell Press, Lancaster, Pa.
38. Doan, C. A., Wilson, H. E., and Wright, C.-S. 1946. *Ohio State Med. J.* **42,** 139–144.
39. Editorial. 1941. *J. Am. Med. Assoc.* **116,** 2169.
40. Frommeyer, W. B., Jr., Spies, T. D., Vilter, C. F., and English, A. 1946. *J. Lab. Clin. Med.* **31,** 643–649.
41. Goldsmith, G. A. 1945. Reported at the AAAS Conference on Vitamins, Gibson Island, July 23–27.
42. Goldsmith, G. A. 1946a. Reported at the AAAS Conference on Vitamins, Gibson Island, July 29–August 2.
43. Goldsmith, G. A. 1946b. Personal communication.
44. Goldsmith, G. A. 1946c. *Federation Proc.* **5,** 232.
44a. Goldsmith, G. A. 1946d. *J. Lab. Clin. Med.* **31,** 1186–1200.
45. Hanes, F. M. 1946. Tice's Practice of Medicine, IV, W. F. Prior, Hagerstown, pp. 171–196.
46. Heinle, R. W., and Welch, A. D. 1946. Personal communication.
46a. Heinle, R. W., and Welch, A. D. 1947. *J. Am. Med. Assoc.* **133,** 739–741.
47. Heinle, R. W., Welch, A. D., and Nelson, E. M. 1946. Reported at the 38th Annual Meeting of the American Society for Clinical Investigation, Atlantic City, May 27.
47a. Hernandez-Beguerie, R. L., and Spies, T. D. 1946. *Am. J. Roentgenol.* **56,** 337–342.
48. Hertz, R. 1945. *Endocrinology* **37,** 1–6.
49. Hertz, R., and Sebrell, W. H. 1944. *Science* **100,** 293–294.
50. Hogan, A. G. 1944. *Nutrition Revs.* **2,** 223.
51. Hogan, A. G., and Parrott, E. M. 1939. *J. Biol. Chem.* **128,** xlvi–xlvii.
52. Hogan, A. G., and Parrott, E. M. 1940. *J. Biol. Chem.* **132,** 507–517.
53. Holmes, E. G. 1945. *Brit. Med. J.* **2,** 561–564.
54. Hurst, A. 1942. *Guy's Hosp. Repts.* **91,** 1–21.

55. Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., Sloane, N. H., and SubbaRow, Y. 1946. *Ann. N.Y. Acad. Sci.* **48**, 265–277.
56. Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., and Slobodkin, N. H. 1944. *Science* **99**, 371.
57. Ingelfinger, F. J. 1943. *New Engl. J. Med.* **228**, 180–184.
58. Isaacs, R., Bethell, F. H., Riddle, M. C., and Friedman, A. 1938. *J. Am. Med. Assoc.* **111**, 2291.
59. Isaacs, R., and Friedman, A. 1938. *Am. J. Med. Sci.* **196**, 718–719.
60. Johnson, B. C. 1946. *J. Biol. Chem.* **163**, 255–259.
61. Johnson, B. C., Hamilton, T. S., and Mitchell, H. H. 1945. *J. Biol. Chem.* **159**, 425–429.
62. Jones, E. 1945. Reported in the Section on Medicine, Southern Med. Assoc., Cincinnati, November 12–15. See Discussion of Goldsmith, G. A. 1946. *Southern Med. J.* **39**, 485–493.
63. Jones, E. and Darby, W. J. 1946. Unpublished observations.
63a. Jones, E., Warden, H. F., and Darby, W. J. 1947. *J. Lab. Clin. Med.* **32**, 387–391.
64. Kaufmann, J., and Schwager, P. G. 1946. *Can. Med. Assoc. J.* **54**, 539–544.
65. Kelly, E. C. 1937. *Med. Classics* **2**, 233–280.
66. Kornberg, A., Daft, F. S., and Sebrell, W. H. 1946. *Science* **103**, 646–648.
67. Krehl, W. A., and Elvehjem, C. A. 1945. *J. Biol. Chem.* **158**, 173–179.
67a. Krehl, W. A., Torbet, N., de la Huerga, J., and Elvehjem, C. A. 1946. *Arch. Biochem.* **11**, 363–369.
68. Langston, W. C., Darby, W. J., Shukers, C. F., and Day, P. L. 1938. *J. Exptl. Med.* **68**, 923–940.
69. Luckey, T. D., Briggs, G. M., Jr., Moore, P. R., Elvehjem, C. A., and Hart, E. B. 1945. *J. Biol. Chem.* **161**, 395–403.
70. McCarrison, R. 1921. Studies in Deficiency Disease, Henry Frowde and Hodder and Stoughton, London, p. 58. (Reproduced in 1945 by Lee Foundation for Nutrition Research, Milwaukee).
71. Mallory, M. E., Mims, V., Totter, J. R., and Day, P. L. 1944. *J. Biol. Chem.* **156**, 317–321.
72. Mims, V., Totter, J. R., and Day, P. L. 1944. *J. Biol. Chem.* **155**, 401–405.
73. Minot, A. S., and Frank, H. E. 1944. *Am. J. Diseases Children* **67**, 371–375.
74. Minot, G. R., and Castle, W. B. 1935. *Lancet* **2**, 319–330.
75. Minot, G. R., and Strauss, M. B. 1943. *Vitamins & Hormones* **1**, 269–291.
76. Mitchell, H. K., Snell, E. E., and Williams, R. J. 1941. *J. Am. Chem. Soc.* **63**, 2284.
77. Moore, C. V. 1946. Personal communication.
78. Moore, C. V., Bierbaum, O. S., Heinle, R. W., and Welch, A D. 1946. *Federation Proc.* **5**, 236.
79. Moore, C. V., Bierbaum, O. S., Welch, A. D., and Wright, L. D. 1945. *J. Lab. Clin. Med.* **30**, 1056–1069.
80. Moore, C. V., Vilter, R., Minnich, V., and Spies, T. D. 1944. *J. Lab. Clin. Med.* **29**, 1226–1255.
81. Napier, L. E. 1938. *Lancet* **2**, 106–107.
82. Norris, L. C., and Heuser, G. F. 1945. *Ann. Rev. Biochem.* **14**, 500–504.
83. O'Dell, B. L., and Hogan, A. G. 1943. *J. Biol. Chem.* **149**, 323–337.
84. Peterson, J. C. 1946. Personal communication.

85. Peterson, J. C., and Dunn, S. C. 1946. *Am. J. Diseases Children* **71,** 252–268.

86. Pfiffner, J. J., Binkley, S. B., Bloom, E. S., Brown, R. A., Bird, O. D., Emmett, A. D., Hogan, A. G., and O'Dell, B. L. 1943. *Science* **97,** 404–405.

87. Pfiffner, J. J., Calkins, D. G., Bloom, E. S., and O'Dell, B. L. 1946. *J. Am. Chem. Soc.* **68,** 1392.

88. Pfiffner, J. J., Calkins, D. G., O'Dell, B. L., Bloom, E. S., Brown, R. A., Campbell, C. J., and Bird, O. D. 1945. *Science* **102,** 228–230.

88a. Pfiffner, J. J. and Hogan, A. G. 1946. *Vitamins & Hormones* **4,** 1–34.

89. Riddle, M. C. 1930. *Arch. Internal Med.* **46,** 417–439.

89a. Saslaw, S., Wilson, H. E., Doan, C. A., Woolpert, O. C., and Schwab, J. L. 1946. *J. Exptl. Med.* **84,** 263–276.

90. Sharp, E. A., and Vonder Heide, E. C. 1944. *Proc. Cent. Soc. Clin. Research* **17,** 41.

91. Sharp, E. A., Vonder Heide, E. C., and Wolter, J. G. 1944. *J. Am. Med. Assoc.* **124,** 734.

92. Shukers, C. F. 1946. Personal communication.

93. Snell, E. E., and Peterson, W. H. 1939. *J. Biol. Chem.* **128,** xciv–xcv.

94. Snell, E. E., and Peterson, W. H. 1940. *J. Bact.* **39,** 273–285.

95. Spies, T. D. 1946a. *J. Am. Med. Assoc.* **130,** 474–477.

96. Spies, T. D. 1946b. *Southern Med. J.* **39,** 634–635.

97. Spies, T. D. 1946c. *Lancet* **1,** 225–228.

97a. Spies, T. D., and Stone, R. E. 1947. *Southern Med. J.* **40,** 46–55.

98. Spies, T. D., Frommeyer, W. B., Jr., Lopez, G. G., Toca, R. L., and Gwinner, G. 1946g. *Lancet* **1,** 883–885.

99. Spies, T. D., Frommeyer, W. B., Jr., Vilter, C. F., and English, A. 1946f. *Blood* **1,** 185–188.

100. Spies, T. D., Lopez, G. G., Menéndez, J. A., Minnich, V., and Koch, M. B. 1946b. *Southern Med. J.* **39,** 30–32.

101. Spies, T. D., Milanes, F., Menéndez, J. A., Koch, M. B., and Minnich, V. 1946c. *J. Lab. Clin. Med.* **31,** 227–241.

102. Spies, T. D., Suárez, R. M., Suárez, R. M., Jr., and Hernandez-Morales, F. 1946d. *Science* **104,** 75–76.

103. Spies, T. D., Vilter, C. F., Cline, J. K., and Frommeyer, W. B. Jr. 1946a. *Southern Med. J.* **39,** 269–270.

104. Spies, T. D., Vilter, C. F., Koch, M. B., and Caldwell, M. H. 1945. *Southern Med. J.* **38,** 707–709.

105. Stokstad, E. L. R. 1943. *J. Biol. Chem.* **149,** 573–574.

106. Stokstad, E. L. R., and Manning, P. D. V. 1938. *J. Biol. Chem.* **125,** 687–696.

107. Suárez, R. M., Spies, T. D., and Suárez, R. M., Jr. 1946. Reported at the 27th Annual Session of the American College of Physicians, Philadelphia, May 17.

107a. Suárez, R. M., Welch, A. D., Heinle, R. W., Suárez, R. M., Jr., and Nelson, E. M. 1946. *J. Lab. Clin. Med.* **31,** 1294–1304.

108. SubbaRow, Y., Hastings, A. B., and Elkin, M. 1945. *Vitamins & Hormones* **3,** 237–296.

109. Totter, J. R. 1945. *Nutrition Revs.* **3,** 351.

110. Villada, R. F. 1942. *Prensa méd. mexicana* **7,** 8.

111. Vilter, C. F., Spies, T. D., and Koch, M. B. 1945. *Southern Med. J.* **38,** 781–785.

112. Vilter, C. F., Vilter, R. W., and Spies, T. D. 1947. *J. Lab. Clin. Med.* **32,** 262–273.

112a. Vilter, R. W. 1943. *Med. Clinics N. America* **27,** 467–481.

113. Waisman, H. A., Rasmussen, A. F., Jr., Elvehjem, C. A., and Clark, P. F. 1943. *J. Nutrition* **26,** 205–218.

114. Watson, C. J., Sebrell, W. H., McKelvey, J. L., and Daft, F. S. 1945. *Am. J. Med. Sci.* **210,** 463–470.

115. Watson, J., and Castle, W. B. 1946. *Am. J. Med. Sci.* **211,** 513–530

116. Welch, A. D., Heinle, R. W., Nelson, E. M., and Nelson, H. V. 1946. *J. Biol. Chem.* **164,** 787–788.

117. Wieder, S. 1944. The Present Status of *Lactobacillus Casei* Factor, Lederle Laboratories, Inc., Pearl River.

118. Wilkinson, J. F., Israëls, M. C. G., and Fletcher, F. 1946. *Lancet* **2,** 156–158.

119. Wills, L. 1934. *Indian J. Med. Research* **21,** 669–681.

120. Wills, L., Clutterbuck, P. W., and Evans, B. D. F. 1937. *Biochem. J.* **31,** 2136–2147.

121. Wills, L., and Evans, B. D. F. 1938. *Lancet* **2,** 416–421.

122. Wintrobe, M. M. 1939. *Am. J. Med. Sci.* **197,** 286–310.

123. Wintrobe, M. M. 1946. Clinical Hematology, 2nd ed., Lea & Febiger, Phila.

124. Wright, L. D., Skeggs, H. R., Welch, A. D., Sprague, K. L., and Mattis, P. A. 1945. *J. Nutrition,* **29,** 289–298.

125. Youmans, J. B., and Patton, E. W. 1946. Reported at the Meeting of the Association of American Physicians, Atlantic City, May 28–29.

126. Zuelzer, W. W., and Ogden, F. N. 1946a. *Proc. Soc. Exptl. Biol. Med.* **61,** 176–177.

127. Zuelzer, W. W., and Ogden, F. N. 1946b. *Am. J. Diseases Children* **71,** 211–243.

The Vitamin Requirements of Chicks[1]

By H. R. BIRD

CONTENTS

Page
I. Introduction . . . 163
II. Vitamin A . . . 165
III. Vitamin D . . . 165
IV. Thiamine . . . 166
V. Riboflavin . . . 166
VI. Pantothenic Acid . . . 166
VII. Niacin, Pyridoxin, Biotin . . . 167
VIII. Vitamin K . . . 167
IX. Choline . . . 167
X. Other Factors . . . 168
XI. Requirements of Other Avian Species . . . 169
XII. Vitamin Content of Purified Diets Currently Used . . . 170
XIII. Summary . . . 171
References . . . 171

I. INTRODUCTION

The chick differs from other commonly used laboratory animals in that its nutritional requirements and processes have been investigated not only to contribute to the science of nutrition but also to permit a more efficient and profitable production of this economically important animal. It has been subjected, therefore, not only to purified diets but also to a large number of modifications of practical or commercial feed mixtures. Furthermore, experiments, have differed as to criterion of effectiveness of the vitamin supplement, duration of test, and genetic background and previous history of the chicks used. This variety of basal diets and methods has not been conducive to unanimous agreement on the chick's quantitative needs. In a recent report on folic acid, Luckey *et al.* (1946a,b) stated, "No definite requirement for this compound can be established since the response to a given amount of folic acid depends upon the type of ration used." If "definite requirement" means a single figure having the same validity as a physical constant, the reviewer agrees heartily with this statement and believes it could be applied just as well to vitamin D, vitamin E, pantothenic acid, niacin, biotin, and choline, and perhaps to all of the required nutrients. Viewed in another way, results such as those just mentioned, obtained under a variety of conditions, should permit greater confidence in stating a

---

[1] Presented at the meeting of the American Chemical Society in Chicago, September, 1946.

"definite requirement," not as one unqualified value, but as a range of values or a minimum value for a given set of conditions.

The chick's requirements for several of the vitamins have been determined under sufficiently various conditions so that they may be stated with some assurance. In the case of other vitamins the stated requirements must be regarded as provisional because of the small number of experiments on which they are based. Sufficient evidence is available upon which to base a schedule of vitamin requirements to serve as a guide in formulating experimental diets, thus facilitating the use of the chick as a laboratory tool.

In 1944 the Committee on Animal Nutrition of the National Research Council published its "Recommended Nutrient Allowances for Poultry" (Cravens *et al.*, 1944; 1946). These allowances included specified margins of safety beyond the requirements agreed upon by a sub-committee after a careful evaluation of the literature. Table I contains a schedule of vitamin requirements derived by subtracting the margins of safety from the allowances recommended. The resulting figures should represent the

TABLE I

Vitamin Requirements of Chicks

(Per 100 g. of diet)

	Calculated from recommended allowances N.R.C. (20)	According to individual investigators
Vitamin A., I.U.	260	134 (66), 140 (62), 150 (55), 160 (53) 160[1] (53), 167[1] (61), 250[1] (4), 250[1] (63)
Vitamin D., A.O.A.C.U.	25	17 (18), 18 (2), 19 (45), 50 (29)
Thiamine hydrochloride, γ	170	80 (1), 150 (35)
Riboflavin, γ	300	290 (46), 300 (5), 300 (6), 360 (10)
Pantothenic acid (Ca salt) γ	1000	660(3),750 (67),1000 (36), 1400 (34)
Niacin, γ	1500	1800 (15)
Pyridoxin hydrochloride, γ	300	300 (16), 400 (32)
Biotin, γ	10	10 (26)
Choline chloride, mg	130	100 (27), 150 (52)
Vitamin K, γ	40	
Vitamin E, γ	....	< 300 (50)
Folic acid, γ	....	25 (43), 55 (56), 100 (17), 100 (33)
p-Aminobenzoic acid, mg[2]	....	7.5 (14)

[1] Vitamin A supplied as carotene of alfalfa meal.

[2] Probably not required when folic acid present.

Figures in parentheses are reference citations.

conclusions of the sub-committee, based on the available literature. The table also contains the figures reported by individual investigators both before and after the appearance of the aforementioned report. Only those reports of individual investigators were included which were stated in, or could be translated into, the units currently used, and which were based on experiments in which the pure vitamins or supplements of known potency were fed at several different levels per unit of diet.

II. VITAMIN A

Any conclusions regarding the requirement for vitamin A are complicated by the two forms in which this vitamin is supplied. However, the difference between the requirements for carotene and for vitamin A as such is not great enough to make this a major complication. The carotene in all the experiments cited in the table was supplied as alfalfa meal and the vitamin A as cod liver oil. The evidence indicates that the latter was a little more effective than the former.

A more serious complication is the possibility that most of the studies on vitamin A requirement have not been continued long enough or have not used sufficiently sensitive criteria to indicate the true requirement. In two of the reports quoted in the table, the statement is made that the requirement increases with age (Record *et al.*, 1937; Tepper and Durgin, 1938). It seems possible that supposedly adequate intakes of vitamin A during the first few weeks of life may permit slow depletion of bodily stores so that a need for a higher dietary level becomes apparent later. Furthermore, Jungherr (1943) has reported that 250 I.U. of vitamin A per chick per day are required to prevent pathologic changes of the nasal mucosa. The vitamin was given in weekly doses so that this result cannot be compared closely with those in the table, but the intake suggested by Jungherr would be at least 4 times the highest figure in the table.

As a result of these considerations, the recommended allowance of the National Research Council was based on a figure near the top of the range of requirements cited in the table rather than on an intermediate value.

All of the tabulated figures for vitamin A were determined with basal diets composed of natural ingredients. It is worthy of note that purified diets, low in natural antioxidants and containing salts of iron and copper, have been shown to lose vitamin A with sufficient rapidity to permit the development of deficiency symptoms even though the vitamin was originally included in considerable excess (Bird and Oleson, 1938).

III. VITAMIN D

The requirement for vitamin D is dependent on the calcium and phosphorus content of the diet and is reduced almost to the vanishing point if very high levels of these elements are fed (Couch *et al.*, 1937; Dols, 1936; Nowotarski and Bird, 1943; Olsson, 1941). The figures given in the table were all determined with diets composed of natural feedstuffs and containing 1.5–2.5% of calcium and 0.9% of phosphorus. Most experimental diets would probably approximate these levels. The exceptionally high figure reported by Heiman and Tighe (1942) was based on the fact that chicks fed this level grew at a slightly greater rate to the age of five weeks and showed a slightly greater degree of calcification than did chicks receiving 30 units. In most studies of vitamin D requirement, conclusions have been based primarily on per cent of bone ash. It is important to note that the chick's requirement for vitamin D cannot be effectively satisfied by supplying vitamin D_2.

IV. THIAMINE

The two discordant values for thiamine requirement were based on different criteria of effectiveness. The lower was found to prevent deficiency symptoms and to support growth. The higher was reported to be adequate for optimal growth. The recommendation of the National Research Council was obviously based on the higher figure. The "rounding-off" incident to the calculations accounts for the slight discrepancy. In both the investigations cited the basal diet was composed of natural feedstuffs some of which were autoclaved to reduce the thiamine content. Lamoreux and Hutt (1939) have reported a breed difference in susceptibility to thiamine deficiency which presumably reflects a difference in requirements.

V. RIBOFLAVIN

Information regarding the requirement of the chick for riboflavin is in a more satisfactory state than is the case for any other vitamin. There is reasonably good agreement among the four reports cited in spite of great dissimilarity among the basal diets. Diets of natural feedstuffs were used by all workers except F. H. Bird *et al.* (1946) who used diets composed in part of purified ingredients and in part of natural ingredients subjected to extraction to reduce their riboflavin content.

Heuser *et al.* (1938) have reported that the riboflavin requirement decreases with increasing age, being 350 γ/100 g. of diet during the second week of life and only 100 γ during the eighth week. In most cases it would not be practical to take advantage of this decrease in formulating laboratory diets.

VI. PANTOTHENIC ACID

The basal diets used in studying pantothenic acid requirements have consisted of natural feedstuffs extracted or heated to reduce their content of the vitamin and supplemented with various crystalline vitamins and concentrates. The most serious disagreement among the reports cited is probably due to the fact that Jukes (1939), who found a requirement of 1400 γ, worked with previously depleted chicks whereas the other investigators did not. Until further research explains the differences among the other three figures, acceptance of the highest of the three would seem to be the conservative solution.

VII. NIACIN, PYRIDOXIN, BIOTIN

Information on the requirements for niacin, pyridoxin, and biotin is based on a very limited number of experiments with diets consisting of synthetic and purified ingredients. Further work is required to define the range of requirements applicable to the varying conditions likely to be encountered in nutritional experiments. Already it has been shown that the niacin requirement of the chick is increased by the presence in the diet of gelatin (Briggs, 1945) or corn (Sarma and Elvehjem, 1946; Scott *et al.*, 1946), and decreased by the presence of tryptophan (Briggs, 1945). The basal diet used in arriving at the requirement of 1800 γ contained 10% of gelatin, and it seems likely that the requirement might be appreciably lower in the case of diets not containing this ingredient.

That unknown factors influence the pyridoxin requirement of chicks is indicated by the report of Lucas *et al.* (1946) that symptoms of pyridoxin deficiency developed in chicks fed a purified diet containing 500 γ of this vitamin/100 g. Since the symptoms disappeared upon the administration of additional pyridoxin, their identity was definitely established.

VIII. VITAMIN K

Experiments on vitamin K have been largely concerned with the size of dose needed to bring about a specified response in deficient chicks. There is very little information that can be interpreted even approximately in terms of requirement per unit of feed. Stamler *et al.* (1943) reported that 1.0–2.0 γ of 2-methyl-1,4-naphthoquinone per day were needed to maintain the prothrombin at a normal level in young White Leghorn chicks. These levels would approximate a range of 10–20 γ/100 g. of feed.

IX. CHOLINE

The requirement for choline was found by Record and Bethke (1942) to be 150 mg./100 g. of a diet composed largely of natural feedstuffs. It was found by Hegsted *et al.* (1941) to be 100 mg./100 g. of a purified diet. Both diets contained casein as a major source of protein. Diets containing proteins of lower methionine content would be expected to show a higher choline requirement.

X. OTHER FACTORS

In addition to the vitamins discussed above, synthetic and purified diets commonly contain folic acid, *p*-aminobenzoic acid, inositol, and vitamin E. Of these, folic acid has been most thoroughly investigated with respect to requirement, probably because it has proved to be the "missing link," the addition of which makes it possible to dispense with crude concentrates of water-soluble factors. It has been suggested, however, that folic acid may function indirectly, at least in part, by stimulating the formation of dietary factors still uncharacterized (Luckey *et al.*, 1946a,b). Reference has already been made to the effect of composition of the diet on folic acid requirement. Luckey *et al.* (1946a,b) found the requirement to be as low as 10 γ/100 g. in a diet high in protein, and as high as 100 γ in a diet high in sucrose or fat. This situation probably accounts for the variable results obtained previously in different laboratories.

Before crystalline folic acid was available, the addition to a purified diet of *p*-aminobenzoic acid up to a level of 7.5 mg./100 g. was reported by Briggs *et al.* (1943) to stimulate growth. This compound does not appear to be essential in diets containing folic acid and, since it forms part of the folic acid molecule, it may have been acting as a partial substitute for folic acid in the earlier experiments.

Purified diets commonly contain 50 mg. of inositol/100 g. because of the report of Hegsted *et al.* (1941) that this compound was essential for the growth of chicks fed such diets. There is no information as to quantitative requirement.

The requirement for vitamin E in diets composed of ordinary feedstuffs was found by Patrick and Morgan (1943) to be less than 300 γ/100 g. Considerably more than this quantity was found by Dam and Glavind (1939) to be required to prevent edema in chicks fed diets that are conducive to this condition. High levels have been included in some purified diets, probably because of the antioxidant properties of this vitamin (Campbell *et al.*, 1944).

Scott *et al.* (1945) reported that either α- or β-pyracin was required in addition to folic acid to promote growth and prevent anemia of chicks fed purified diets. This was contradicted by Hutchings *et al.* (1946). There is no information as to quantitative requirements for this factor.

Evidence was presented recently for the existence of an unidentified factor required for optimal growth of chicks fed soybean meal as the major source of protein (Hill *et al.*, 1944; Patton *et al.*, 1946; Rubin and Bird, 1946). It does not appear to be required as a supplement to purified diets containing casein.

XI. REQUIREMENTS OF OTHER AVIAN SPECIES

The mature pigeon has long been used as a laboratory animal, and the growing turkey poult and duckling are gaining favor as subjects of nutritional research. It would seem also that the recently attained importance of embryonated eggs in medical science would justify greater attention to the dietary factors needed by the hen for the production of viable embryos. For these reasons the available information on the vitamin requirements of these animals has been summarized in Table II and compared with the chick's requirements.

TABLE II

Vitamin Requirements of Avian Species

(Per 100 g. of diet)

	Pigeon, maintenance	Chicken, reproduction (20)	Chicken growth (20)	Turkey, growth	Duck, growth
Vitamin A., I.U.	....	440	260	530 (20)	
Vitamin D, A.O.A.C.U.	....	65	25	80 (44)	30 (24)
Thiamine hydrochloride, γ	125 (65)	....	170		
Riboflavin, γ	....	240	300	370 (20)	300 (23)
Pantothenic acid (Ca salt) γ	....	1400	1000		
Niacin, γ	....	....	1500	5000 (12)	
Pyridoxin hydrochloride γ	....	300	300	....	250 (28)
Biotin, γ	....	13	10		
Choline chloride, mg.	....	....	130	170 (20)	

Figures in parentheses are reference citations.

The requirements of the mature chicken for reproduction were calculated from the Recommended Nutrient Allowances for Poultry (Cravens *et al.*, 1944). Comparison of these figures with the requirements of the chick shows that greater quantities of vitamins A and D and pantothenic acid are needed for reproduction. The high requirement for vitamin D is undoubtedly associated with the very active calcium metabolism incident to egg production. There is no obvious reason for the high requirements of the other two vitamins.

In view of the extensive use of the pigeon in nutritional research, the almost complete lack of information as to its quantitative requirements is surprising. Its use has been confined largely to studies of thiamine. For the most part, the vitamin has been administered therapeutically rather than prophylactically. The report of Swank and Bessey (1941) indicates that the thiamine requirement per unit of feed is in the same range as that of the chick. Lee and Hogan (1942) reported that the pigeon needed riboflavin, pantothenic acid, pyridoxin and possibly niacin, but quantitative requirements were not determined.

The turkey poult requires more of all five of the vitamins investigated than does the chick. This situation has been ascribed to the relatively rapid early growth of the turkey. However, this explanation is inadequate, since the duckling, which grows more rapidly than the turkey, corresponds to the chick in its requirement for the three vitamins studied. The turkey poult's requirement for vitamin D is especially interesting because it is more sensitive than that of the chick to the quantity of inorganic phosphorus in the diet and to the form of vitamin D employed (Matterson *et al.*, 1946). The high requirement of the turkey for niacin was determined, like that of the chicken, with a diet containing 10% of gelatin. The turkey poult requires pantothenic acid (Lepkovsky *et al.*, 1945), pyridoxin (Bird *et al.*, 1943), biotin (Patrick *et al.*, 1941) and folic acid (Richardson *et al.*, 1945), but the quantitative requirements have not been determined.

XII. VITAMIN CONTENT OF PURIFIED DIETS CURRENTLY USED

To compare the vitamin content of some purified diets recently described in the literature with the requirements discussed above, three such diets were selected and their vitamin contents tabulated in Table III. The diets selected were the ones used by Scott *et al.* (1945) in the study of pyracin, by Campbell *et al.* (1944) in the study of vitamin B_c, and by Briggs *et al.* (1945) in the study of vitamins B_{10} and B_{11}. It is

TABLE III

Vitamin Content of Synthetic Chick Diets Compared with Requirements

(Per 100 g. of diet)

	Requirements	Diets		
		(13)	(17)	(60)
Vitamin A, I.U.	260	[1]	1600	1000
Vitamin D, A.O.A.C.U.	25	[1]	160	200
Thiamine hydrochloride, γ	170	300	400	500
Riboflavin, γ	300	600	800	1000
Pantothenic acid (Ca or Na salt), γ	1000	2000	1100	1630
Niacin, γ	1500	5000	2000	3000
Pyridoxin hydrochloride, γ	300	400	600	500
Biotin, γ	10	20	[1]	10
Choline chloride, mg	130	150	200	200

[1] By dropper.

Figures in parentheses are reference citations.

immediately apparent that the former two groups of investigators used a rather large excess of vitamins A and D, thiamine and riboflavin, and that Briggs *et al.* used a large excess of niacin. A large excess of vitamin A probably is needed to compensate for rapid destruction, and if the vitamins A and D are supplied in a single supplement, as they were in the experiments of Scott *et al.* (1945), this necessitates a large excess of vitamin D. The reasons for the high levels of the water-soluble vitamins are not apparent. There is no evidence that a superabundance of thiamine, riboflavin or niacin would influence results of these or other experiments. However, it has been demonstrated (Bird and Rubin, 1946) that with a certain diet composed of natural ingredients supplemented with synthetic choline the growth of chicks was stimulated by additions of either pantothenic acid or pyridoxin in excess of the accepted requirement even though the diet itself contained supposedly adequate quantities of these vitamins. There would appear to be some reason for avoiding large excesses of vitamins except in studies intended to ascertain the effects of such excesses. Until these effects are learned there is reason to be conservative in establishing margins of safety between the best available figures for requirements and the quantities supplied in laboratory diets.

XIII. SUMMARY

A review of the literature on the vitamin requirements of the growing chick shows that the needs for vitamins A and D, riboflavin and pantothenic acid, and perhaps folic acid, have been investigated under sufficiently various conditions so that the range of values determined should be adequate for conditions likely to be encountered in the use of the chick as a laboratory animal. Further study of the causes of the apparent variations in requirement for vitamin A and pantothenic acid is desirable. Requirements for thiamine, niacin, pyridoxin, biotin, vitamin K, and choline can be established provisionally on the basis of present information. Further study is needed to determine the effect of varying experimental conditions.

Some laboratory diets contain quantities of certain vitamins greatly exceeding the requirements. There is evidence that, in some cases, results may be influenced in an unexpected manner by a large excess of one or more nutrients.

The vitamin requirements of the chick have been summarized and compared with those of other avian subjects of nutritional research.

REFERENCES

1. Arnold, A., and Elvehjem, C. A. 1938. *J. Nutrition* **15**, 403–410.
2. Baird, F. D., and Greene, D. J. 1935. *Poultry Sci.* **14**, 70–82.

3. Bauernfeind, J. C., Norris, L. C., and Heuser, G. F. 1942. *Poultry Sci.* **21,** 142–146.
4. Bearse, G. E., and Miller, M. W. 1937. *Poultry Sci.* **16,** 34–38.
5. Bethke, R. M., and Record, P. R. 1942. *Poultry Sci.* **21,** 147–154.
6. Bird, F. H., Asmundson, V. S., Kratzer, F. H., and Lepkovsky, S. 1946. *Poultry Sci.* **25,** 47–51.
7. Bird, F. H., Kratzer, F. H., Asmundson, V. S., and Lepkovsky, S. 1943. *Proc. Soc. Exptl. Biol. Med.* **52,** 44–45.
8. Bird, H. R., and Oleson, J. J. 1938. *Proc. Soc. Exptl. Biol. Med.* **38,** 870–871.
9. Bird, H. R., and Rubin, M., 1946. *Poultry Sci.* **25,** 87–89.
10. Bolton, W. 1944. *J. Agr. Sci.* **34,** 198–206.
11. Briggs, G. M., Jr. 1945. *J. Biol. Chem.* **161,** 749–750.
12. Briggs, G. M., Jr. 1946. *J. Nutrition* **31,** 79–84.
13. Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B. 1945. *J. Biol. Chem.* **158,** 303–312.
14. Briggs, G. M., Jr., Luckey, T. D., Mills, R. C., Elvehjem, C. A. and Hart, E. B. 1943. *Proc. Soc. Exptl. Biol. Med.* **52,** 7–10.
15. Briggs, G. M., Jr., Mills, R. C., Elvehjem, C. A., and Hart, E. B. 1942. *Proc. Soc. Exptl. Biol. Med.* **51,** 59–61.
16. Briggs, G. M., Jr., Mills, R. C., Hegsted, D. M., Elvehjem, C. A., and Hart, E. B. 1942. *Poultry Sci.* **21,** 379–383.
17. Campbell, C. J., Brown, R. A , and Emmett, A. D. 1944. *J. Biol. Chem.* **152,** 483–484.
18. Carver, J. S., Robertson, E. I., Brazie, D., Johnson, R. H., and St. John, J. L. 1934. *Wash. Agr. Expt. Sta. Bull.* **299.**
19. Couch, J. R., Fraps, G. S., and Sherwood, R. M. 1937. *Poultry Sci.* **16,** 106–108.
20. Cravens, W. W., Almquist, H. J., Bethke, R. M., Norris, L. C., and Titus, H. W. 1944; revised, 1946. Recommended Nutrient Allowances for Poultry. National Research Council.
21. Dam, H., and Glavind, J. 1939. *Skand. Arch. Physiol.* **82,** 299–316.
22. Dols, M. J. L. 1936. *Arch. néerland. physiol.* **21,** 554.
23. Fritz, J. C., Archer, W., and Barker, D. 1939. *Poultry Sci.* **18,** 449–454.
24. Fritz, J. C., Archer, W., and Barker, D. 1941. *Poultry Sci.* **20,** 151–154.
25. Hegsted, D. M., Briggs, G. M., Jr., Mills, R. C., Elvehjem, C. A., and Hart, E. B. 1941. *Proc. Soc. Exptl. Biol. Med.* **47,** 376–377.
26. Hegsted, D. M., Mills, R. C., Briggs, G. M., Jr., Elvehjem, C. A., and Hart, E. B. 1942. *J. Nutrition* **23,** 175–179.
27. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B. 1941. *J. Biol. Chem.* **138,** 459–466.
28. Hegsted, D. M., and Rao, M. N 1945. *J. Nutrition* **30,** 367–374.
29. Heiman, V., and Tighe, L. W. 1942. *Poultry Sci.* **21,** 511–514.
30. Heuser, G. F., Wilgus, H. S., Jr., and Norris, L. C. 1938. *Poultry Sci.* **17,** 105–108.
31. Hill, F. W., Scott, M. L., Norris, L. C., and Heuser, G. F. 1944. *Poultry Sci.* **23,** 253–255.
32. Hogan, A. G., Richardson, L. R., Patrick, H., O'Dell, B. L., and Kempster, H. L. 1940, 1941. *Poultry Sci.* **19,** 352; **20,** 180–183.
33. Hutchings, B. L., Oleson, J. J., and Stokstad, E. L. R. 1946. *J. Biol. Chem.* **163,** 447–453.
34. Jukes, T. H. 1939. *J. Biol. Chem.* **129,** 225–231.

35. Jukes, T. H., and Heitman, H., Jr. 1940. *J. Nutrition* **19,** 21–30.
36. Jukes, T. H., and McElroy, L. W. 1943. *Poultry Sci.* **22,** 438–441.
37. Jungherr, E. 1943. *Conn. Agr. Expt. Sta. Bull.* **250.**
38. Lamoreux, W. F., and Hutt, F. B. 1939. *J. Agr. Research* **58,** 307–316.
39. Lee, J. G., and Hogan, A. G. 1942. *Mo. Agr. Expt. Sta. Research Bull.* **342.**
40. Lepkovsky, S., Bird, F. H., Kratzer, F. H., and Asmundson, V. S. 1945. *Poultry Sci.* **24,** 335–339.
41. Lucas, H. L., Heuser, G. F., and Norris, L. C. 1946. *Poultry Sci.* **25,** 137–142.
42. Luckey, T. D., Moore, P. R., Elvehjem, C. A., and Hart, E. B. 1946a. *Proc. Soc. Exptl. Biol. Med.* **62,** 307–312.
43. Luckey, T. D., Moore, P. R., Elvehjem, C. A., and Hart, E. B. 1946b. *Science* **103,** 682–684.
44. Matterson, L. D., Scott, H. M., and Singsen, E. P. 1946. *J. Nutrition* **31,** 599–608.
45. Murphy, R. R., Hunter, J. E., and Knandel, H. C. 1936. *Pa. Agr. Expt. Sta. Bull.* **334.**
46. Norris, L. C., Wilgus, H. S., Jr., Ringrose, A. T., Heiman, V., and Heuser, G. F. 1936. *Cornell Agr. Expt. Sta. Bull.* **660.**
47. Nowotarski, J. S., and Bird, H. R. 1943. *Poultry Sci.* **22,** 72–78.
48. Olsson, N. 1941. Studien über die Verwendbarkeit wachsender Küken zur Bestimmung des antirachitischen Effekts von Vitamin D und Ultraviolettem Licht. Gleerupska Unweritetsbokhandeln, Lund.
49. Patrick, H., Boucher, R. V., Dutcher, R. A., and Knandel, H. C. 1941. *Proc. Soc. Exptl. Biol. Med.* **48,** 456–458.
50. Patrick, H., and Morgan, C. L. 1943. *Poultry Sci.* **22,** 397–398.
51. Patton, A. R., Marvel, J. P., Petering, H. G., and Waddell, J. 1946. *J. Nutrition* **31,** 485–495.
52. Record, P. R., and Bethke, R. M. 1942. *Poultry Sci.* **21,** 271–276.
53. Record, P. R., Bethke, R. M., and Wilder, O. H. M. 1937. *Poultry Sci.* **16,** 25–33.
54. Richardson, L. R., Hogan, A. G., and Kempster, H. L. 1945. *J. Nutrition* **30,** 151–157.
55. Ringrose, R. C., and Norris, L. C. 1936. *Poultry Sci.* **15,** 390–396.
56. Robertson, E. I., Daniel, L. J., Farmer, F. A., Norris, L. C., and Heuser, G. F. 1946. *Proc. Soc. Exptl. Biol. Med.* **62,** 97–101.
57. Rubin, M., and Bird, H. R. 1946. *J. Biol. Chem.* **163,** 387–392; **163,** 393–400.
58. Sarma, P. S., and Elvehjem, C. A. 1946. *Poultry Sci.* **25,** 39–40.
59. Scott, H. M., Singsen, E. P., and Matterson, L. D. 1946. *Poultry Sci.* **25,** 303–304.
60. Scott, M. L., Norris, L. C., Heuser, G. F., and Bruce, W. F. 1945. *J. Biol. Chem.* **158,** 291–298.
61. Sherwood, R. M. 1939. *Proc., 7th World's Poultry Cong., Cleveland* 123–126.
62. Sherwood, R. M., and Fraps, G. S. 1936. *Texas Agr. Expt. Sta. Bull.* **528.**
63. Sherwood, R. M., and Fraps, G. S. 1940. *Texas Agr. Expt. Sta. Bull.* **583.**
64. Stamler, F. W., Tidrick, R. T., and Warner, E. D. 1943. *J. Nutrition* **26,** 95–103.
65. Swank, R. L., and Bessey, O. A. 1941. *J. Nutrition* **22,** 77–89.
66. Tepper, A. E., and Durgin, R. C. 1938. *N. H. Agr. Expt. Sta. Bull.* **310.**
67. Waisman, H. A., Mills, R. C., and Elvehjem, C. A. 1942. *J. Nutrition* **24,** 187–198.

Vitamin Requirements of the Mouse[1]

By HAROLD P. MORRIS

CONTENTS

Page
I. Introduction 175
II. Thiamine. 176
III. Riboflavin 180
IV. Pantothenic Acid 182
V. Pyridoxine. 185
VI. Biotin and Folic Acid 188
VII. Niacin and Choline 189
VIII. Inositol 189
IX. Ascorbic Acid. 189
X. Vitamins A and D. 190
XI. Vitamin E 190
XII. Mouse Growth Factor in Proteins. 191
XIII. Vitamin Analogues 191
  1. Pantoyltaurine. 191
  2. Riboflavin Analogue 192
  3. Niacin Analogues. 192
  4. Thiamine Analogues 192
  5. α-Tocopherol and Vitamin K Analogues. 193
XIV. Summary 193
References 194

I. INTRODUCTION

The mouse is closely related to the rat taxonomically, yet the vitamin requirements of the two species are not sufficiently alike to permit the direct application to the mouse of those vitamin requirements established for the rat.

One of the first points to keep clearly in mind is the relative growth rate of the two species (Morris, 1944). This is illustrated in Fig. 1, which shows the percentage increase in weight of rats (curve A) and mice (curve B) from birth to 70 days of age. The two species grow at the same rate relative to their birth weight for the first 4 weeks. After that the rat increases in weight much more rapidly than the mouse. By 70 days of age the rat has multiplied its birth weight 48 times while the mouse has multiplied it only 16 times. The percentage increase in weight relative to weaning weight of mice (Curve D) and rats (Curve C) from 3 weeks of age up to 10 weeks of age is shown. The two species increase at about the same rate between 3 and 5 weeks of age, while after

---

[1] Presented in part at a symposium on the "Vitamin Requirements of Laboratory Animals," Div. Agr. and Food Chem., A. C. S., Chicago, September, 1946.

5 weeks, the rat increases more rapidly than the mouse. The relative increase in weight of the mouse is not significantly greater than the rat during the first 10 weeks of life.

The two-week period following the weaning at about 24 days of age is the period of most rapid growth for the mouse. It has been the experience in our laboratories that the growth rate of mice is more rapid on synthetic diets if feeding is started when the animals weigh 10–12 g., than when the mice are younger and smaller. Growth is rapid for the following 5 weeks under normal conditions. When a normal mouse reaches 7–8 weeks of age, its growth curve levels off. Dietary regimens in which growth rate is a measure of vitamin requirements should be carried out during that period.

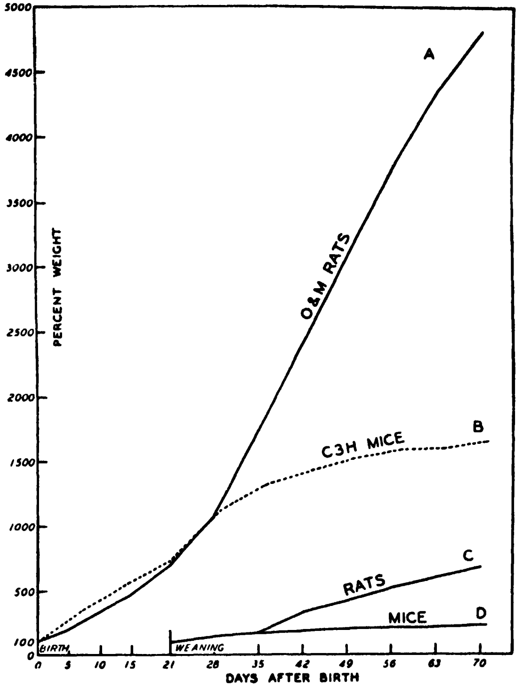


FIG. 1.—Relative growth rates of mice and rats.

II. THIAMINE

One of the first clinical manifestations of thiamine deficiency in experimental mice is failure to maintain adequate caloric intake. In the complete absence of dietary thiamine, depletion is so rapid that the animals die before they have developed other typical symptoms (Swank and Bessey, 1941). Thus, when the food consumption of C3H mice was limited to the amount consumed by animals on a thiamine-deficient diet (Morris and Dubnik, 1944), as shown in Fig. 2, survival in both groups was the same, *i.e.*, 3–4 weeks. This observation was confirmed by Jones *et al.* (1945) for albino mice deficient in thiamine.

The first quantitative study of the thiamine requirements of young weanling albino mice was made by Hauschildt (1942) using a basal diet low in fat. He established the following daily requirements: maintenance of body weight 5 γ, suboptimal growth 7 γ or 8 γ, and essentially normal growth 10 γ. Using a diet high in fat (22%), Morris and Dubnik (1944) found young, weanling, C3H mice required an average of 4.1 γ daily for maintenance of body weight, and 5–6 γ for maximum growth, while adult mice required less than 5–6 γ daily for maintenance. These results suggest that the requirement of mice on a high fat diet is somewhat less than on the low fat diet.

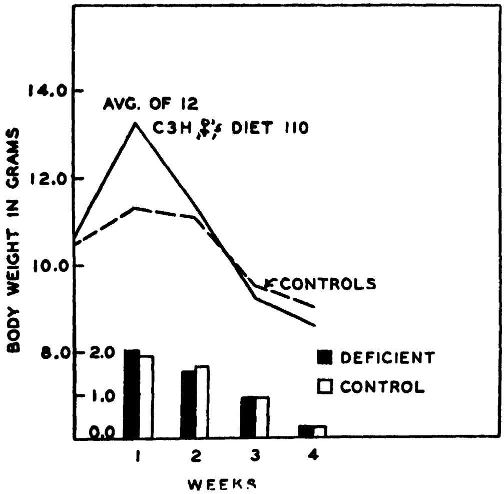


Fig. 2.—Average body weight in acute thiamine deficiency. Diet 110 is deficient in thiamine.

To maintain the caloric intake of thiamine-deficient mice Morris and Dubnik (1944) forcibly fed the basal diet to animals whose thiamine stores had been exhausted. While maintaining their body weight these mice did not survive any longer than did the animals which were allowed to eat the deficient diet *ad libitum*.

Morris *et al.* (unpublished), in a paired feeding experiment with C3H mice, produced a condition which may be called chronic thiamine deficiency. This condition was produced by addition of 1 γ of thiamine/g. of food to the basal diet. This amount of thiamine is inadequate for the continued growth and well-being of the mouse. The average weight-response of mice on the thiamine-deficient diet (curve A) and the response of the controls receiving 10 times more thiamine but allowed the same amount of food (curve B), is shown in Fig. 3. The gain for the first 5–6 weeks was almost identical in both groups. The loss in weight was parallel for the next 3–4 weeks, except that the controls were always slightly heavier at any given time. This experiment demonstrates that the body weight response was mostly due to the amount of food consumed.

The low-thiamine group began to show severe clinical symptoms of thiamine deficiency between 8–11 weeks. These symptoms were characterized by violent convulsions (especially when the animal was held for a few seconds by the tail) and a tendency to move in a circular motion. In some cases the animals would roll over and over, somewhat like the "cartwheel" turning of the severely thiamine-deficient pigeon. The mice survived from 2–9 days after the onset of clinical symptoms. Brain hemorrhages involving the reticular formation in the medulla were frequent (Fig. 4). Prickett (1934) reported similar lesions in the rat.

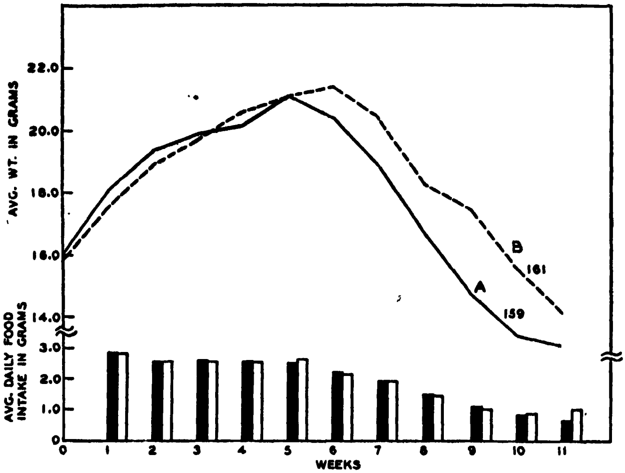


FIG. 3.—Average body weight of mice on 1 γ of thiamine (Curve A) and 10 γ of thiamine (Curve B) per g. of food.

Muscle lesions, presenting an appearance of fine silvery streaks grossly, appeared in the skeletal muscles in most of the deficient mice. Microscopically, this lesion appeared as a necrosis of the muscle fibers (Fig. 5). The rectus capitis group of muscles was most frequently involved. Testicular degeneration occurred in all the deficient mice. The control group ingesting the same amount of basal diet but receiving an excess amount of thiamine did not develop brain lesions or testicular damage, and only rarely showed the muscle changes. In acute thiamine

FIG. 4.—Brain hemorrhages (black areas) in mice deficient in thiamine (× 6).

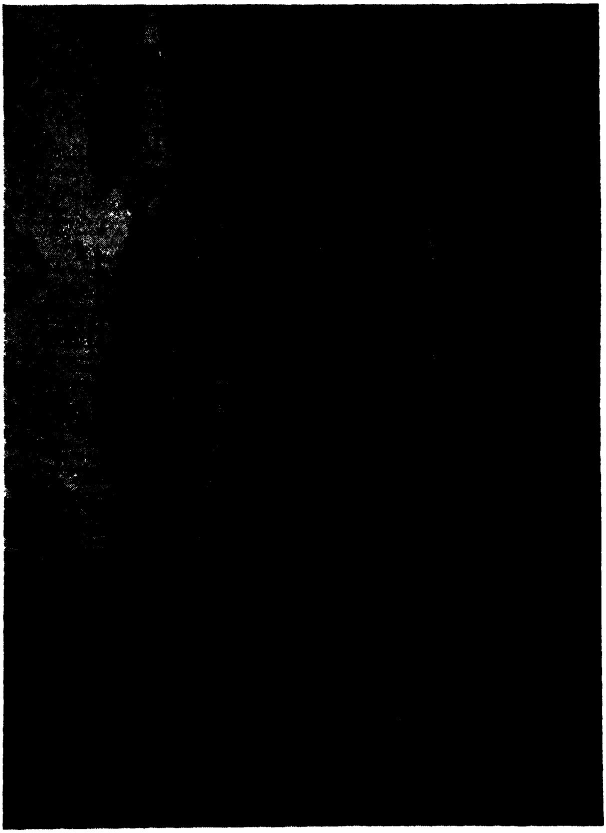
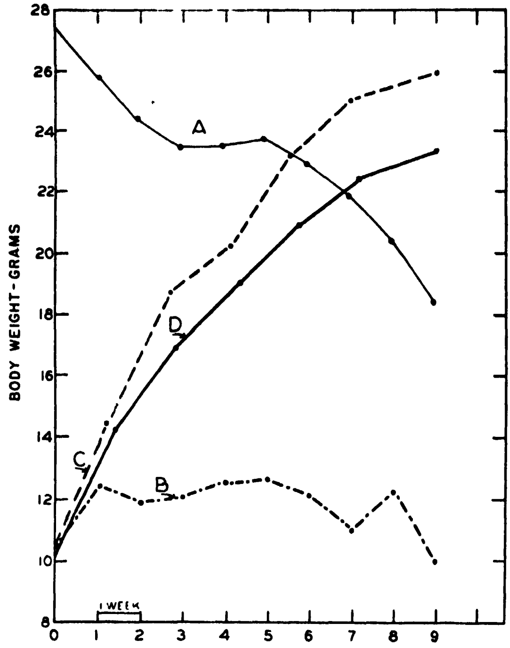


FIG. 5.—Muscle necrosis in chronic thiamine deficiency (× 285).

deficiency, however, brain hemorrhages occasionally developed, but no muscle lesions were observed.

III. RIBOFLAVIN

Morris and Robertson (1943) found that adult mice lost weight promptly and continuously and survived about 9 weeks when fed a basal diet containing 0.4–0.6 γ riboflavin/g. of food (Fig. 6, curve A). Young mice failed to grow on this diet after the initial body stores of riboflavin had been exhausted, maintained their weight about 5 weeks, then declined in weight until death (Fig. 6, curve B). Acute ariboflavinosis in these young mice resulted in atrophic and hyperkeratotic dermatosis which was occasionally followed by a dermatitis, some myelin degenera-



AVERAGE DAILY FOOD INTAKE-GRAMS

A 2.6 2.5 2.6 2.5 2.4 2.6 2.6 2.6 2.6
B 1.7 1.6 1.6 1.6 1.5 1.6 1.6 1.8 1.8
C 2.0 2.6 2.3 2.5 2.6 2.6

FIG. 6.—Average body weight and average food intake of normal mice on diets deficient in, and supplemented with, riboflavin, and body weight of mice on stock diet. Curve A, adult deficient mice; curve B, young deficient mice; curve C, young mice on supplemented basal diet; curve D, young stock mice. Daily food intake values refer to curves A, B and C, respectively. From Morris and Dubnik (1944).

tion in the spinal cord, and a keratitis of the eye (Lippincott and Morris, 1942). Riboflavin deficiency did not have much influence on food intake in either young or adult mice (Morris and Robertson, 1943). However, after depletion, and when the deficient diet was supplemented with an abundant amount of riboflavin, the food consumption of young mice increased. Deficient adult animals lost weight, yet their food intake remained constant throughout the experimental period (Fig. 6, food intake A). This suggests a definite trend toward a lowered efficiency of food utilization. Sure (1941) made a similar observation on rats deficient in riboflavin, which was also not associated with any marked anorexia in the animals during the terminal stages of ariboflavinosis. The gross clinical picture of loss of hair, development of cracks and fissures in the skin (especially in the region of the snout), and exudate on the conjunctiva of depleted animals, is indicated in Fig. 7.

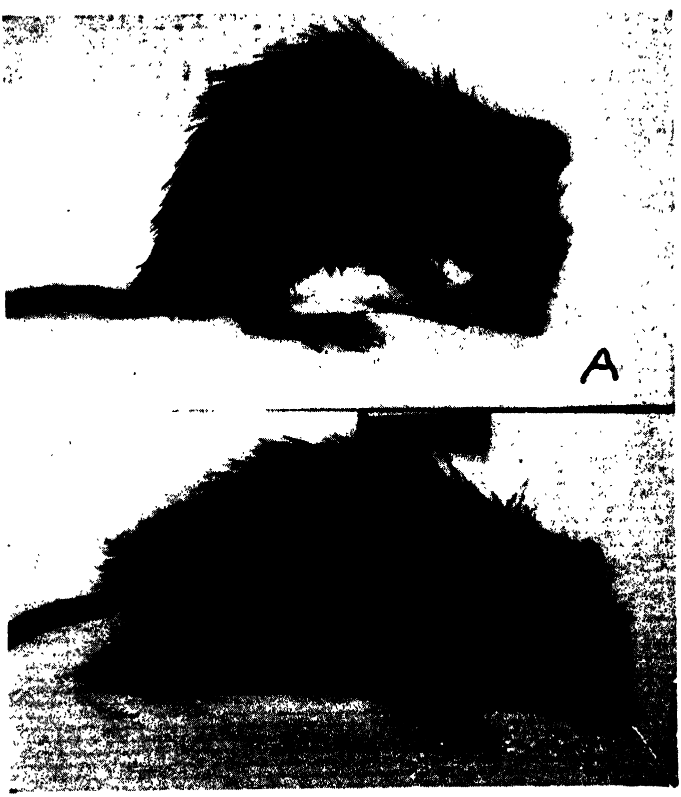


Fig. 7.—Clinical appearance of an adult mouse in chronic partial ariboflavinosis. A, Cutaneous lesions with marked distortion of ears. At rest animal remains in this hunched position. B, Dragging of the hind legs by the same animal when walking.

The maintenance requirement for adult mice, was found to be about 1.5 γ/g. of food ingested, or approximately 4 γ/mouse/day.

IV. PANTOTHENIC ACID

The first effect of a pantothenic acid deficiency in weanling C3H strain mice was a growth failure manifested about 1 week after the institution of the deficient regimen (Morris and Lippincott, 1941). The young mice gradually lost weight (Fig. 8, Lowest curve), and their survival did not extend much beyond 6 weeks. Jones *et al.* (1945), working with young albino mice, obtained survivals for 67 days, with 21% of the mice surviving for 205 days. There is no apparent explanation for this longer survival. Adult mice in our experiments showed a very rapid loss in weight on the pantothenic acid-deficient regimen, and a survival of only 6–8 weeks (Fig. 9), but rapid restoration of lost weight when pantothenic acid was added.

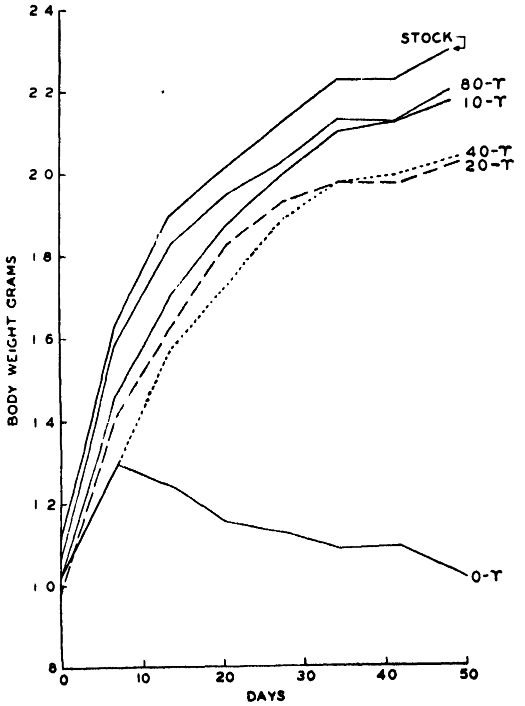


FIG. 8.—Average body weight curves of young mice supplemented with different levels of pantothenic acid.

Adult mice after 4 weeks on the deficient regimen showed characteristic changes in the hair (Morris and Lippincott, 1941). Denuding occurred chiefly on the ventral surface, flanks and hind legs. Frequently preceding, but occasionally following, depilation a keratotic, desquamative dermatosis which presented a scaly, chalk-like appearance grossly (Fig. 10A) developed. When pantothenic acid was administered, recovery took place in about 10 days (Fig. 10B). Fig. 11 shows the typical squatting position of a pantothenic acid-deficient mouse. During the latter part of the deficient state partial paralysis affecting the hind legs was noted. Histologic findings (Lippincott and Morris, 1941) demonstrated myelin degeneration of the spinal cord, posterior roots, and sciatic nerves, while the brain remained intact. The hemorrhagic adrenal cortical necrosis observed in rats (Unna, 1940; Daft *et al.*, 1940; Salmon and Engel, 1940; Mills *et al.*, 1940) on diets deficient in pantothenic acid did not occur in C3H mice (Lippincott and Morris, 1941).

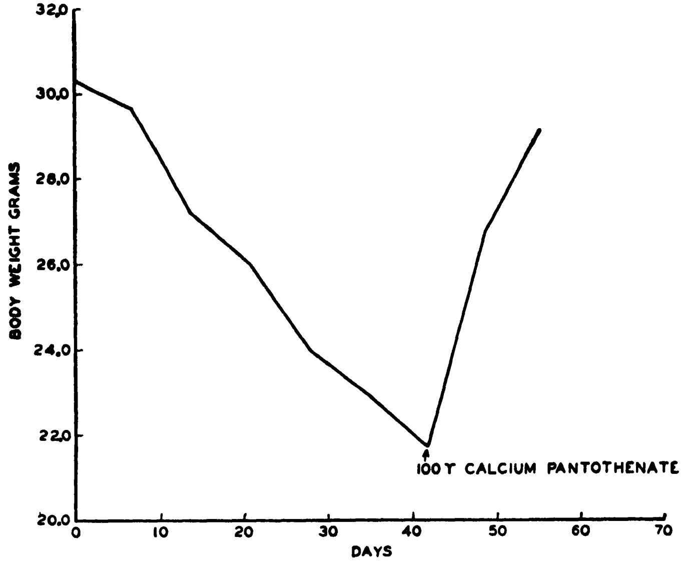


FIG. 9.—Loss of body weight in pantothenic acid deficiency in adult mice and the effect of pantothenic acid supplementation on body weight.

Graying of the hair in C57 black mice deficient in pantothenic acid was reported by György and Poling (1940). The fur became silvery gray or brownish, similar in color to the fur of the house mouse, after the mice had been on the deficient regimen from 3 to 5 weeks. Definite effect on the depigmentation of the fur and practical cure with 50–100 γ of calcium pantothenate daily were observed in 3–5 weeks. However, after several months on this synthetic diet containing adequate pantothenic acid there was observed a scaly dermatosis, a thinning of the pelt, and a change in the pigment of the fur. The addition of a highly purified biotin concentrate resulted in definite and rapid improvement in the pigment of the fur. This effect was noted only when the biotin was given during the period of secondary changes in the fur and after the initial achromothrichia had been relieved by pantothenic acid.

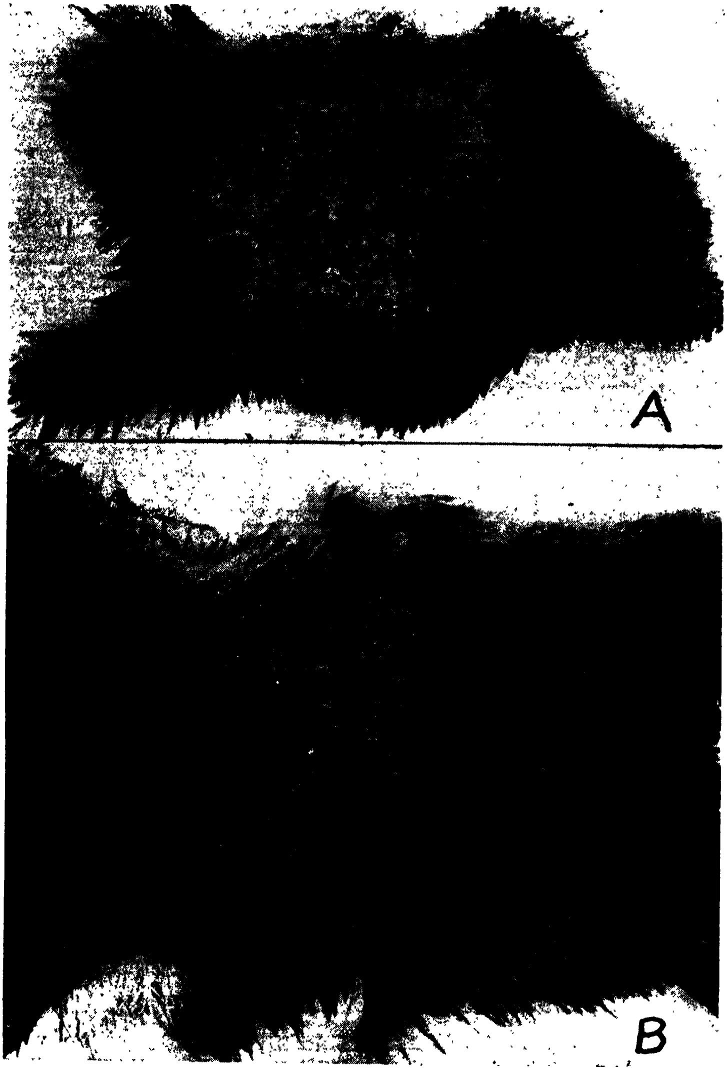


FIG. 10.—A, Severe dermatosis due to pantothenic acid deficiency. B, Recovery 10 days after pantothenic acid administration. From Lippincott and Morris (1941).

The extensive alopecia in mice of the type observed by Norris and Hauschildt (1940) on a diet low in pantothenic acid and inositol, was cured by Martin (1941) by the daily addition of 150 γ pantothenic acid, but the graying of the fur of Rockland strain black mice was not completely cured by pantothenic acid. Woolley (1942) observed a deficiency of inositol in the tissues of mice in the absence of pantothenic acid from the diet. When pantothenic acid was added to this diet, the normal content of the tissues was quickly restored.

FIG. 11.—Characteristic squatting position of a pantothenic acid-deficient mouse.

The requirement of the mouse for pantothenic acid has been determined on two strains: albino weanling mice (Sandza and Cerecedo, 1941) and strain C3H mice (Morris and Lippincott, 1941). By daily injection of calcium pantothenate Sandza and Cerecedo (1941) concluded that the mouse requires about 30 γ of *d*-calcium pantothenate daily. This value was confirmed by Morris and Lippincott (1941) by feeding the vitamin, indicating that animals with pigmented coats do not require more pantothenic acid than albinos. Furthermore, calcium pantothenate could be administered orally or subcutaneously with equal effect.

V. PYRIDOXINE

Adult mice require pyridoxine for maintenance of body weight (Morris and Dubnik, unpublished). Adult mice in our laboratories fed a diet deficient in pyridoxine lost weight rapidly and died in 7–8 weeks (Fig. 12, curve A). Pyridoxine added to this diet brought about prompt recovery of weight (Fig. 12, curve B).

Miller and Baumann (1945) observed a paralysis in the hind legs in the pyridoxine-deficient young mice but no dermatitis. No dermatitis in pyridoxine-deficient mice was observed in our laboratory. These observations are in marked contrast to pyridoxine deficiency in the rat where a specific symmetrical dermatitis or acrodynia occurs. The clinical picture of adult C3H mice surviving 8 weeks on pyridoxine-deficient diets is characterized by lifeless, necrotic tails, ears covered by a scaly encrustation, and loss of hair, especially in encrusted areas over the forehead and shoulders, underside of the neck and along the ventral surface. The hind legs are carried forward with some difficulty and the animals have the appearance of walking stiff-legged.

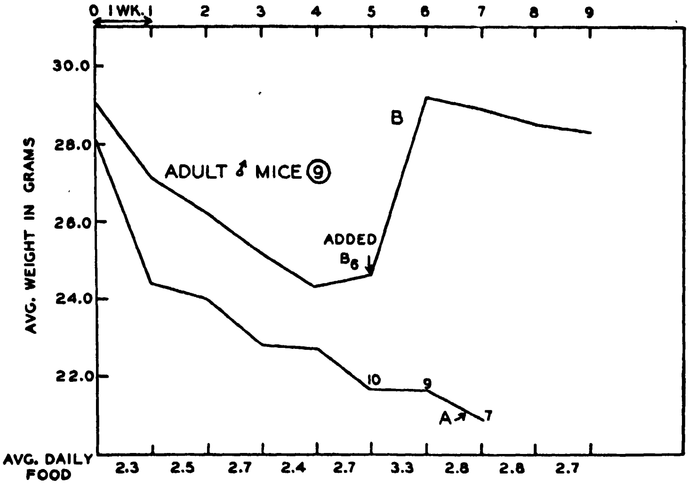


FIG. 12.—Average body weight of young and adult mice on a pyridoxine-deficient diet and the effect of pyridoxine supplementation on the body weight of adult mice.

Young weanling mice on pyridoxine-deficient diets containing 18–20% casein have been shown by Miller and Baumann (1945) and by Morris and Dubnik (unpublished) to make very good growth gains when supplemented with 1 γ pyridoxine/g. of food (Fig. 13, curve A). No growth improvement resulted with higher levels of supplementation (Fig. 13, curve B). Morris and Dubnik found that the supplementation of the diet with 0.5 γ pyridoxine/g. of food (Fig. 13, curve C) gave a half-normal growth response, while Miller and Baumann (1946) obtained essentially normal growth at the 0.5 γ level (Fig. 13, curve D). No difference in food intake was observed by Morris and Dubnik when the diet was supplemented with either 0.5 γ or 1.0 γ of pyridoxine. The data of Miller and Baumann (1945) show that the growth rate and survival of weanling mice receiving 2 γ or more of pyridoxine/g. of food was unaffected by variations of 10–60% of dietary casein (Curves B,D,F of

Fig. 14). There was a progressive shortened survival of mice receiving no pyridoxine as the dietary casein was being increased from 10% to 60% (curves A,C,E of Fig. 14).

The requirement of mice for pyridoxine was increased 3–4 times, however, by raising the casein level to 60%. Growth, and the excretion of a chromogen determined as xanthurenic acid, were within normal limits only by feeding 2 γ or more of pyridoxine/g. of food. Mice fed a pyridoxine-free diet containing 10% casein supplemented with DL-tryptophan equivalent to a 45% casein diet, excreted more urinary chromogen, consumed more food, appeared in better health, and survived longer, than mice on the 45% casein diet. Pyridoxine restored growth and chromogen excretion to normal. Addition of histidine or tyrosine to the diet of pyridoxine-deficient mice was not deleterious. The addition of DL-phenylalanine, however, increased the excretion of a chromogen not identical with that excreted after the addition of tryptophan.

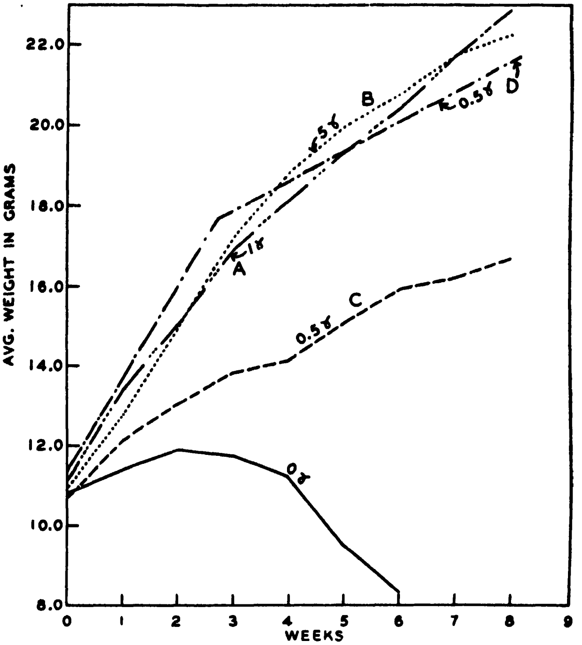


FIG. 13.—Average body weight of mice receiving 18–20% casein diets when supplemented with different levels of pyridoxine.

Miller and Baumann (1946) have compared the relative activity of molar equivalents of pyridoxine, pyridoxal and pyridoxamine for albino mice. They used growth and the level of xanthurenic acid excretion as criteria of potency at 0.75 γ and 3.0 γ levels of pyridoxine per gram of food. They concluded that pyridoxamine and pyridoxal were less active for mice than pyridoxine, especially on high casein diets.

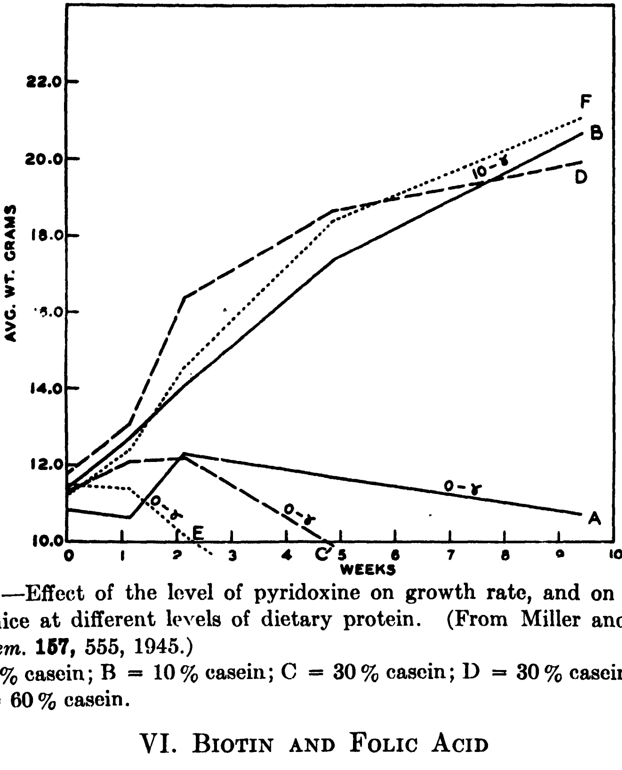


FIG. 14.—Effect of the level of pyridoxine on growth rate, and on survival, of weanling mice at different levels of dietary protein. (From Miller and Baumann, *J. Biol. Chem.* **157**, 555, 1945.)

A = 10% casein; B = 10% casein; C = 30% casein; D = 30% casein; E = 60% casein; F = 60% casein.

VI. BIOTIN AND FOLIC ACID

Nielsen and Black (1944) obtained some stimulation of growth in albino mice when the basal diet was supplemented with either biotin or folic acid concentrate (eluate factor) or with both. Very little, if any, growth response was obtained when the basal ration contained sulfasuxidine and was then supplemented with either biotin or the folic acid concentrate. An alopecia developed in the group receiving the basal diet plus folic acid concentrate which was partially alleviated by biotin.

Very poor growth was secured on their basal diet between 2 and 8 weeks and according to the authors only mild symptoms of biotin- and folic acid-deficiency were produced. They interpreted this as indicating inadequate synthesis of these two vitamins by the intestinal flora of the mouse. These experiments, however, are not very clear-cut, especially because of the poor growth response of their mice to the basal ration. Other investigators with basal rations similar to those used by Nielsen and Black have obtained good growth in mice. Additional studies are needed to establish the biotin and pteroylglutamic acid requirements of mice.

VII. Niacin and Choline

These substances are usually included in diets for mice. No studies have been reported on the niacin requirement of mice. It has been observed by White and Cerecedo (1946) in choline deficiency that young mice develop kidney lesions by 14 days after weaning only when they are given a choline-deficient diet beginning prior to weaning. Mice proved more resistant to the development of renal damage on choline-deficient diets which produce hemorrhagic kidneys in rats.

VIII. Inositol

A dietary antialopecia factor was described and identified as inositol by Woolley (1940; 1941). Woolley (1942) found that pantothenic acid in the diet resulted in increasing the inositol concentration in tissues of young mice which recovered from an alopecia, but not in those animals which failed to recover. In the absence of pantothenic acid from the diet of mice fed inositol, the tissues were deficient in this substance, and inclusion of pantothenic acid in the diet resulted in a normal inositol content of the animals. The spontaneous cures of alopecia in mice reported by Woolley probably involve synthesis of inositol by microorganisms in the alimentary canal.

IX. Ascorbic Acid

Woolley and Krampitz (1943) have succeeded in producing a scurvy-like condition in mice by feeding 10% glucoascorbic acid (2,3-enediol-*d*-glucoheptone-1,4-lactone), a homologue of ascorbic acid, in a purified diet. The condition was characterized by loss in weight, diarrhea, and hemorrhage, and death within 2 weeks. However, when 10% glucoascorbic acid was added to diets consisting of natural foodstuffs, a similar condition could not be produced. Woolley and Krampitz were unable to cure the symptoms in mice with ascorbic acid but when Woolley (1944a) produced a similar condition in guinea pigs by feeding glucoascorbic acid it could be prevented with ascorbic acid. From the results he obtained on guinea pigs he concluded that an ascorbic acid deficiency had been produced in both species.

These results however were not confirmed by Banerjee and Elvehjem (1945) using rats, chicks and guinea pigs. They could not produce the hemorrhages, although diarrhea was observed, which could not be prevented with ascorbic acid. The inclusion of 2% of a vitamin C-free liver powder to the diet which contained either 10% glucoascorbic or ascorbic acid, permitted normal growth of rats in spite of a moderate diarrhea. These authors suggest that unfavorable effects of glucoascorbic acid might be due, in part, to a modification of the intestinal flora resulting in the failure to produce some undetermined factor normally supplied by the intestinal flora and which in their experiments was supplied by liver powder. One must remember that an extremely large quantity of glucoascorbic acid was used in both experiments and therefore the results reported might be misleading. Reyniers (1946) has reported that ascorbic acid stimulated the growth of mice reared in a germ-free environment. This stimulation was noted both before and after the usual age for weaning. It is not yet clear whether ascorbic acid is produced by the intestinal flora, or whether some other factor is produced by microorganisms which influences the *in vivo* synthesis of ascorbic acid in mice.

X. VITAMINS A AND D

There is a lack of experimental studies on the quantitative requirements of mice for vitamins A and D. If one estimates the vitamin A requirement as derived from studies of other species on the basis of body weight as Rosenberg (1942) did, one obtains a minimum daily value, which does not allow for any storage, of 1 γ of β-carotene or 0.1 of–0.2 γ vitamin A/adult mouse/day. For growth and reproduction at least 3 times that value of vitamin A and 5 times the minimum of β-carotene should be allowed.

Beard and Pomerene (1929) reported that the mouse reacts in the same way as the rat to a deficiency of vitamin D. They found that mice develop rickets more quickly than rats and were cured in a shorter time.

XI. VITAMIN E

Bryan and Mason (1940) found that female mice on a vitamin E-deficient diet became sufficiently depleted in vitamin E after rearing 1–2 litters to develop the typical resorption gestation observed in rats. The histopathologic changes observed in the resorption sites of mice resembled in every way those encountered in the vitamin E-deficient rat. Bryan and Mason (1940) were unable to induce sterility in the male mouse after 100–400 days on the deficient regimen. No instance of testicular degeneration was found, under experimental conditions which, for the male rat, will show changes in from 35–50 days. Goettsch (1942) reported that growth of the male mouse is not retarded on a synthetic, vitamin E-low diet, nor was the average age of sexual maturity affected either by the presence or absence of vitamin E. The quantitative requirements of the female mouse for vitamin E have been studied by several investigators. Bryan and Mason (1940) found 350 γ of α-tocopherol was the minimum amount necessary to give all positive responses at the first pregnancy. Goettsch (1942) estimated that 0.5–1 mg. of α-tocopherol was required to permit birth of young to 85% of mothers deficient in vitamin E, when given as a single dose at the beginning of gestation. Goettsch compared the α-tocopherol requirements of rats and mice on the basis of surface area of the two species and concluded that they were similar.

Pappenheimer (1942) has reported muscular dystrophy and hyalin necrosis of muscle fibers in only 15% of young mice, whereas, under similar conditions, 90% of young rats are affected.

Cerecedo and Vinson (1944) believe that a relationship exists between vitamin E and protein metabolism because muscular paralysis and incoordination in young mice was almost entirely prevented by increasing the dietary casein from 17 to 25%, or by addition of 20 mg. α-tocopherol/kg. of diet.

XII. Mouse Growth Factor in Proteins

A substance called strepogenin by Woolley has been reported to have growth-stimulating properties for a variety of bacteria as well as for mice (Sprince and Woolley, 1945; Woolley, 1946a). This substance has not yet been identified. Woolley (1946a) regards this unidentified substance, which is present in a number of proteins, as an integral part of the protein molecule possessing a peptide-like structure. Strepogenin is included in this discussion because of its growth-promoting property when fed in diets containing nitrogen from pure amino acid mixtures or from acid hydrolyzates of proteins with added tryptophan and cystine. The crude material is effective in amounts of 0.01–0.1% of the ration. A mouse would be expected to consume from 0.5–4 mg. of the material daily. Womack and Rose (1946) also have reported the presence of an unidentified substance in proteins essential for maximum growth of rats fed diets containing mixtures of purified amino acids. Whether the workers in these two laboratories are dealing with the same substance remains to be established.

XIII. Vitamin Analogues

1. *Pantoyltaurine*

Snell *et al.* (1943) reported that a disease resembling pantothenic acid-deficiency could be produced in laboratory strains of mice by the oral administration of pantoyltaurine (N-(α,α-dihydroxy-β,β-dimethylbutyryl)-taurine). This compound is the sulfur analogue of pantothenic acid and is structurally related to it. The authors reported cessation of growth in 3–4 weeks, roughened hair, the appearance of porphyrin deposits in the whiskers, and behavior symptoms similar to those described for mice deficient in pantothenic acid (Sandza and Cerecedo, 1941). The authors concluded that it is probable that pantoyltaurine interferes specifically with the metabolism of pantothenic acid in animals, a hypothesis similar to that advanced by McIlwain (1940, 1942) that, in bacteria, pantoyltaurine, by virtue of its structural similarity, competes in the utilization of pantothenic acid. Other workers with mice, hamsters (Woolley and White, 1943a), and rats (Unna, 1943) were unable to secure evidence of any antagonism between pantoyltaurine and pantothenic acid.

2. Riboflavin Analogue

Woolley (1944b) fed 2,4-dinitro-7,8-dimethyl-10-ribityl-5,10-dihydrophenazine, the phenazine analogue of riboflavin, to mice and produced a condition somewhat similar to a riboflavin deficiency which was prevented by feeding 1 mg. of riboflavin/g. of ration. The deficiency usually did not prove fatal even when large amounts of the phenazine analogue were fed.

3. Niacin Analogues

Woolley (1945a) has reported feeding mice 3-acetylpyridine, a structural analogue of niacin, in which the acetyl group has been substituted for the carboxyl group of niacin. Feeding 2 mg. or more daily to mice produced several of the symptoms typical of niacin deficiency in dogs and humans. Two *per cent* niacin added to the diet prevented the development of symptoms and growth was normal. Once symptoms developed, however, they could not be cured by feeding niacin. Woolley (1945b) also showed DL-tryptophan protected mice receiving 3-acetylpyridine. Woolley and White (1943a) reported that feeding pyridine-3-sulfonic acid, another analogue of niacin, had no affect on mice. Woolley (1946b) prepared an extract from corn which produced symptoms somewhat similar to those produced by 3-acetylpyridine. These symptoms were preventable or curable by 0.2% of niacinamide in the diet. The question arises as to whether this extract contains a pyridine analogue of niacin.

4. Thiamine Analogues

Woolley and White (1943b) have reported a condition in mice having some characteristics of thiamine deficiency and produced by the administration of pyrithiamine, the pyridine analogue of thiamine, which could be prevented or cured by sufficient amounts of thiamine.

Soodak and Cerecedo (1946) report growth inhibition of mice on thiamine deficient diets by daily injections of oxythiamine.

5. *α-Tocopherol and Vitamin K Analogues*

It has been observed by Woolley (1945c) that either the feeding or introperitoneal injection of *dl-α*-tocopherolquinone to pregnant mice induced clinical symptoms related to those observed in α-tocopherol deficiency. Oral doses of 100 mg. daily of the quinone beginning on the 5th or 6th day of pregnancy caused no detectable symptoms till the 3rd week. From the 14th–19th day the rapid increase in weight was arrested, followed about a day later by extensive vaginal bleeding. Complete resorption took place. If treatment was stopped, complete recovery of the animal occurred with ability to reproduce and bear subsequent litters. Daily oral administration of massive doses of *dl-α*-tocopherol acetate simultaneously with the quinone did not prevent the resorptions. Twenty γ daily of 2-methyl-1,4-naphthoquinone (vitamin K) did prevent the vaginal hemorrhages as well as the resorptions, and allowed the normal production of young. The quinone had no influence on the prothrombin time of mouse plasma. The full mechanism of the action of this compound is not yet apparent, although the α-tocopherol quinone has structural similarities to both vitamins E and K.

XIV. SUMMARY

Thiamine, riboflavin, pantothenic acid, and pyridoxine are essential for growth of young mice and maintenance of weight of adult mice. There appear to be normally present in the intestinal flora of mice, microorganisms which can synthesize inositol, biotin, ascorbic acid, and pteroylglutamic acid, so that, while these substances are probably essential for the mouse, they may be produced in sufficient amounts by the organisms of the digestive tract when the diet contains an adequate supply of the other vitamins, especially those of the vitamin B group. Thiamine requirements appear to be decreased when the diet is high in fat.

The specific acrodynia which develops in pyridoxine-deficient rats apparently does not occur in the mouse. Pyridoxine requirements are increased by high levels of casein in the diet. Pyridoxamine and pyridoxal are less potent than pyridoxine for growth and prevention of xanthurenic acid excretion on high casein diets.

The choline and niacin requirements have not yet been established for mice.

The male mouse apparently grows equally well with or without α-tocopherol and does not develop testicular degeneration in vitamin E deficiency. The female mouse undergoes resorption phenomena identical with those occurring in the female rat deficient in vitamin E. Muscular dystrophy occurs less frequently in the young vitamin E-deficient mouse than in the young rat.

Analogues of thiamine, riboflavin, and pyridoxine have been prepared which produce clinical evidences of deficiencies in mice, which are preventable or curable by the addition of large amounts of their respective vitamins.

Insofar as different strains of mice have been used to establish the vitamin requirements of this species they appear to respond in the same way.

Much progress has been made during the last five or six years in establishing quantitative values for some of the vitamins required by mice. We do not consider these amounts as final values, because they have been worked out under a very limited set of conditions. We believe, however, that they can serve as very useful sign-posts to guide future research on this species.

REFERENCES

1. Banerjee, S., and Elvehjem, C. A. 1945. *Proc. Soc. Exptl. Biol. Med.* **60,** 4–7.
2. Beard, H. H., and Pomerene, E. 1929. *Am. J. Physiol.* **89,** 54–57.
3. Bryan, W. L., and Mason, K. E. 1940. *Am. J. Physiol.* **131,** 263–267.
4. Cerecedo, L. R., and Vinson, L. J. 1944. *Federation Proc.* **3,** 55.
5. Daft, F. S., Sebrell, W. H., Babcock, S. H., Jr., and Jukes, T. H. 1940. *U. S. Pub. Health Service, Pub. Health Rept.* **55,** 1333–1337.
6. Goettsch, M. A. 1942. *J. Nutrition* **23,** 513–523.
7. György, P., and Poling, C. E. 1940. *Proc. Soc. Exptl. Biol. Med.* **45,** 773–776.
8. Hauschildt, J. D. 1942. *Proc. Soc. Exptl. Biol. Med.* **49,** 145–147.
9. Jones, J. H., Foster, C., Dorfam, F., and Hunter, G. 1945. *J. Nutrition* **29,** 127–136.
10. Lippincott, S. W., and Morris, H. P. 1941. *J. Natl. Cancer Inst.* **2,** 39–46.
11. Lippincott, S. W., and Morris, H. P. 1942. *J. Natl. Cancer Inst.* **2,** 601–610.
12. Martin, G. J. 1941. *Science* **93,** 422–423.
13. McIlwain, H. 1940. *Brit. J. Exptl. Path.* **21,** 136–147.
14. McIlwain, H. 1942. *Brit. J. Exptl. Path.* **23,** 95–102.
15. Miller, E. C., and Baumann, C. A. 1945. *J. Biol. Chem.* **157,** 551–562.
16. Miller, E. C., and Baumann, C. A. 1946. *J. Biol. Chem.* **159,** 173–183.
17. Mills, R. C., Shaw, J. H., Elvehjem, C. A., and Phillips, P. H. 1940. *Proc. Soc. Exptl. Biol. Med.* **45,** 482–484.
18. Morris, H. P. 1944. *J. Natl. Cancer Inst.* **5,** 115–142.
19. Morris, H. P., and Dubnik, C. S. 1944. *Proc. Am. Chem. Soc.* New York, Sept. 11–14.
20. Morris, H. P., and Dubnik, C. S. Unpublished.
21. Morris, H. P., Dunn, T. B., and Dubnik, C. S. Unpublished.
22. Morris, H. P., and Lippincott, S. W. 1941. *J. Natl. Cancer Inst.* **2,** 29–38.
23. Morris, H. P., and Robertson, W. v. B. 1943. *J. Natl. Cancer Inst.* **3,** 479–489.
24. Nielsen, E. and Black, A. 1944. *J. Nutrition* **28,** 203–207.
25. Norris, E. R., and Hauschildt, J. 1940. *Science* **92,** 315–317.

26. Pappenheimer, A. M. 1942. *Am. J. Path.* **18,** 169–175.
27. Prickett, C. O. 1934. *Am. J. Physiol.* **107,** 459–470.
28. Reyniers, J. A. 1946. Germ-free Life Studies. Lobund Reports 1, Univ. Press, Notre Dame.
29. Rosenberg, H. R. 1942. The Chemistry and Physiology of Vitamins. Interscience, New York.
30. Salmon, W. D., and Engel, R. W. 1940. *Proc. Soc. Exptl. Biol. Med.* **45,** 621–623.
31. Sandza, J. G., and Cerecedo, L. R. 1941. *J. Nutrition* **21,** 609–615.
32. Snell, E. E., Chan, H., Spiridanoff, S., Way, E. L., and Leake, C. D. 1943. *Science* **97,** 168.
33. Soodak, M., and Cerecedo, L. R. 1946. *Proc. Am. Chem. Soc.* New York, p. 26B, Sept. 9.
34. Sprince, H., and Woolley, D. W. 1945. *J. Am. Chem. Soc.* **67,** 1734–36.
35. Sure, B. 1941. *J. Nutrition* **22,** 295–301.
36. Swank, R. L., and Bessey, O. A. 1941. *J. Nutrition* **22,** 77–89.
37. Unna, K. 1940. *J. Nutrition* **20,** 565–576.
38. Unna, K. 1943. *Proc. Soc. Exptl. Biol. Med.* **54,** 55–57.
38a. White, E. A., and Cerecedo, L. R. 1946. *Proc. Am. Chem. Soc.* 23–24B, April 14.
39. Womack, M., and Rose, W. C. 1946. *J. Biol. Chem.* **162,** 735–736.
40. Woolley, D. W. 1940. *J. Biol. Chem.* **136,** 113–118.
41. Woolley, D. W. 1941. *J. Biol. Chem.* **139,** 20–34.
42. Woolley, D. W. 1942. *J. Exptl. Med.* **75,** 277–284.
43. Woolley, D. W. 1944a. *Federation Proc.* **3,** 97.
44. Woolley, D. W. 1944b. *J. Biol. Chem.* **154,** 31–37.
45. Woolley, D. W. 1945a. *J. Biol. Chem.* **157,** 455–459.
46. Woolley, D. W. 1945b. *J. Biol. Chem.* **162,** 179–180.
47. Woolley, D. W. 1945c. *J. Biol. Chem.* **159,** 59–66.
48. Woolley, D. W. 1946a. *J. Biol. Chem.* **162,** 383–388.
49. Woolley, D. W. 1946b. *J. Biol. Chem.* **164,** 773–774.
50. Woolley, D. W., and Krampitz, L. O. 1943. *J. Exptl. Med.* **78,** 333–339.
51. Woolley, D. W., and White, A. G. C. 1943a. *Proc. Soc. Exptl. Biol. Med.* **52,** 106–108.
52. Woolley, D. W., and White, A. G. C. 1943b. *J. Biol. Chem.* **149,** 285–289.

The Properties of the Growth and Adrenocorticotropic Hormones[1]

BY CHOH HAO LI AND HERBERT M. EVANS

CONTENTS

Page

I. Introduction 198

II. Growth Hormone 199

  1. Methods of Assay 199

    a. Body Growth of Normal Female Rats 199

    b. Body Growth of Hypophysectomized Female Rats 200

    c. Tibia of Hypophysectomized Rats 200

  2. Method of Isolation 201

    a. Historical 201

    b. The Procedure 202

    c. Homogeneity Studies 203

  3. Physical and Chemical Properties 206

    a. Isoelectric Point and Molecular Weight 206

    b. Analytical Data 207

    c. Diffusion and Viscosity 207

    d. Stability 207

  4. Biological Properties 208

    a. Body Growth 208

    b. Nitrogen Retention 211

    c. Stimulation of Cartilage Proliferation 213

    d. Diabetogenicity 213

III. Adrenocorticotropic[2] Hormone 215

  1. Methods of Assay 215

    a. Historical 215

    b. The Repair Test 216

    c. The Maintenance Test 216

  2. Methods of Isolation 216

    a. Swine Hormone 218

    b. Sheep Hormone 218

  3. Physical and Chemical Properties 220

    a. Isoelectric Point 220

    b. Molecular Kinetic Data 220

    c. Some Analytic Data 220

    d. Solubility 220

    e. Stability 220

    f. Effect of Various Reagents 221

  4. Biological Properties 221

    a. Inhibition of Growth 221

    b. Effect on the Gonads of Male Rats 222

    c. Effect on Carbohydrate Metabolism 224

    d. Effect on Lymphoid Tissues and Serum Proteins 225

---

[1] Part of the material in this paper was presented at the first Laurentian Hormone Conference held at Mont Tremblant, Quebec, Canada, September 1944.

[2] See footnote 2, page 233.

Page
e. Effect on Cholesterol and Ascorbic Acid Content of the Adrenal 226
References 227

I. INTRODUCTION

The biological defects which follow hypophysectomy in a mammal and the repair of these defects by crude extracts of the anterior hypophysis or by implantation of anterior hypophyseal tissue have furnished the securest ground for belief in the secretion of hormones by the anterior pituitary. There has been long debate as to the number of hormones secreted by the gland but adequate evidence has established the fact that there are at least six hormones identifiable as individual substances in extracts of anterior pituitary tissue—namely, follicle-stimulating, interstitial-cell stimulating (luteinizing), lactogenic, thyrotropic, adrenocorticotropic and growth hormones. Of these hormones, four have already been isolated in chemically pure form. In addition, the claim has been made that the follicle-stimulating hormone has been obtained in "biologically pure" state (Greep, van Dyke *et al.*, 1940) and this hormone appears to be chemically distinct from all others (Fraenkel-Conrat, Simpson *et al.*, 1940a). Thyrotropic hormone (Ciereszko, 1945) has been highly purified but not yet isolated.

These hormones are protein in nature. In 1937, White, Catchpole *et al.* announced the crystallization of the lactogenic hormone (prolactin) but not until 1942, was the crystalline protein shown to be a homogeneous substance and identified as the hormone (White, Bonsnes *et al.*, 1942). Since this hormone was the first to be indubitably isolated (Li, Lyons *et al.*, 1940a, 1940b, 1941a, 1941b; White, Bonsnes *et al.*, 1942) its chemistry has been most extensively explored and one of the most interesting things which has come out of this exploration has been the establishment of the fact that the hormone isolated from glands of different mammalian species is not an identical substance. For instance, ox prolactin has a higher tyrosine content than that of the sheep hormone (Li, Lyons *et al.*, 1940b). A more remarkable difference was observed in the interstitial-cell stimulating hormone isolated from sheep (Li, Simpson *et al.*, 1940a, 1940b, 1942a) and swine (Shedlovsky, Rothen *et al.*, 1940) pituitaries. On the other hand, the adrenocorticotropic hormone obtained either from sheep (Li, Simpson *et al.*, 1942b, 1943) or from swine (Sayers, White *et al.*, 1943) glands appears to be an identical substance, although porcine glands have a higher hormonal content. We have also to remember that the hormones thus far identified are derived from *extracts* of the pituitary. The question as to the actual number of hormones *secreted* by the gland is still far from clear. In the case of one of the gonadotropins it has been postulated (Fraenkel-Conrat, Simpson *et al.*, 1940b) that the extracted interstitial cell-stimulating hormone is a precursor of the actual secreted hormone. Differences in the adrenocorticotropic potency in the *fresh* and *stored* gland have also been interpreted as indicating a difference in the extracted and secreted hormones (Fraenkel-Conrat, Simpson *et al.*, 1940c). A final answer to such concepts will have to wait upon the isolation and characterization of the hormones actually encountered in the blood stream.

It is well known that many metabolic processes are attributed to the effects of anterior hypophyseal hormones (Long, 1943), particularly the growth and adrenocorticotropic hormones. Biological studies, to be described later, suggest that the metabolic changes resulting from the administration of the growth hormone may involve an entirely different process from that provoked by the adrenocorticotropic hormone. Chemically, these two substances are also very different; the growth hormone is insoluble in water, whereas the adrenocorticotropic hormone is a water-soluble protein. The latter is heat-stable and the former is thermolabile.

II. THE GROWTH HORMONE

1. *Methods of Assay*

Three methods of bioassay are currently employed for the determina tion of growth hormone potency. Two of them are based on the body growth of normal or hypophysectomized female rats and have been widely used in growth hormone research. The third method has been adequately explored only recently and appears to be specific; it depends on the rapid increase in width of the uncalcified cartilage of the epiphyseal disc of a long bone of a young hypophysectomized animal; the proximal epiphysis of the tibia of an hypophysectomized immature rat can be advantageously chosen for such measurements.

a. Body Growth of Normal Female Rats. The use of normal plateaued female rats, 5-6 months of age and weighing from 220 to 280 g. for the assay of growth hormone fractions was first suggested by Evans and Simpson (1931). Such rats, although they have reached growth stasis—the so-called growth plateau—can be induced to grow readily by the administration of the growth hormone. In actual practice, a hormonal preparation to be assayed was injected intraperitoneally or subcutaneously for 20 days (17 injections) into groups each of which consisted of at least 6 normal plateaued female rats. A dose level that causes an increase in body weight of from 40 to 60 g. in this period is most reliably chosen for such an assay (Evans, Uyei *et al.*, 1939). It has been found that a straight line relationship exists between the logarithm of the dose level and the response in body weight (Bulbring, 1938; Marx, Simpson *et al.*, 1942a).

Although normal "plateaued" female rats possess the advantages of being more resistant to the toxic effects of crude preparations and being more easily available, their notably lower sensitivity to the hormone and the longer period of injection required have limited their use.

b. Body Growth of Hypophysectomized Female Rats. It is generally agreed that quantitative assays of pituitary hormones should be carried out in hypophysectomized animals. Smith clearly demonstrated in 1927 that the growth of hypophysectomized rats recurs promptly after pituitary implants or the injection of extracts of the anterior pituitary. For the routine assay of growth hormone preparations in this laboratory (Evans, Uyei *et al.*, 1939), a group of at least 8 hypophysectomized female rats, 28–30 days of age at operation and 10–14 days postoperative, receive 9 intraperitoneal injections in 10 days. When the weight gain is plotted against the logarithm of the dose, a straight line is also obtained (Marx, Simpson *et al.*, 1942a). Hypophysectomized rats have been shown to respond to much smaller doses of the growth hormone than do normal plateaued females. For accurate results the age of the animals and the length of the postoperative period must be uniform; older animals are less responsive.

c. Tibia of Hypophysectomized Rats. The influence of the pituitary on the growth of the skeleton has been known for some time (Evans and Long, 1921a; Dott and Fraser, 1923; Handelman and Gordon, 1930) but the specific effects of growth hormone on the epiphyseal cartilages of hypophysectomized animals have been described only recently (Freud, Levie *et al.*, 1939; Ray, Evans *et al.*, 1941). Kibrick, Becks *et al.* (1941) concluded that these effects were as follows: "Hypophysectomy rapidly initiates a loss in the dimensions of the epiphyseal plate, despite the fact that growth of cartilage and bone may continue for a short time in the young animal after the removal of the pituitary. This loss in thickness reflects the initial disturbance of the equilibrium that normally exists between chondrogenesis and osteogenesis. Administration of growth hormone rapidly restores the dimensions of the cartilage plate by stimulating, first, chondrogenesis and then osteogenesis until an equilibrium is reestablished." Based on these premises Evans, Simpson *et al.* (1943a) proposed a new method for bioassay of the pituitary growth hormone using the width of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats as the index of hormonal stimulation. This method was found approximately three times as sensitive as that dependent upon body growth. When it was tested using other pituitary preparations it was found highly specific for the growth hormone (Marx, Simpson *et al.*, 1944).

The procedure requires the use of hypophysectomized female rats, 26–28 days of age at operation, 12–13 days postoperative. The hormone preparation was administered intraperitoneally once daily for 4 days. The autopsy was made 24 hours after the last injection; the right tibia of each animal was split and fixed in neutral formol. Routine staining with $AgNO_3$ and $Na_2S_2O_3$ was used. The uncalcified portion of the epiphysis was then measured under the microscope with a calibrated eyepiece micrometer.

Fig. 1 presents the tibia of a "control" rat as compared with that of an animal injected with 50 γ of growth hormone. Evans, Simpson *et al.* (1943) also found that a plot of the width of the uncalcified cartilage against the logarithm of the hormone dose gives a straight line.

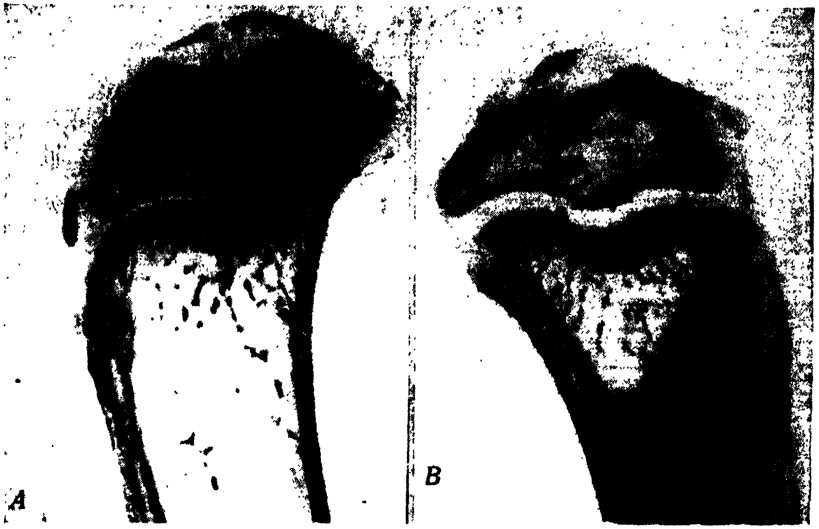


FIG. 1.—Proximal ends of tibias longitudinally split and stained with $AgNO_3$ from hypophysectomized rats. A. Untreated control. B. Treated with growth hormone.

2. Method of Isolation

a. Historical. The first experimental demonstration of the growth-promoting action of the anterior pituitary as carried out by Evans and Long (1921b) utilized a simple saline extract. Later it was found advantageous to prepare growth-promoting extracts by alkaline solutions. Such alkaline solutions may be made from NaOH (Evans and Simpson, 1925), $Ba(OH)_2$ (Evans, Cornish *et al.*, 1929), $Ca(OH)_2$ (Fraenkel-Conrat, Meamber *et al.*, 1940), or NH_4OH (Fevold, Lee *et al.*, 1940). From these extracts, Teel (1929), and van Dyke and Wallen-Lawrence (1930) were able to show that the growth hormone activity is precipitated out as a globulin in the presence of sodium sulfate. This fact led Evans, Uyei *et al.* (1939) to use ammonium sulfate as a precipitating agent for the hormone. They found that growth-promoting activity is in the precipitate which occurs between 0.2 and 0.5 saturated ammonium sulfate. This precipitate, though it contained the growth hormone, was not freed from other active components. In 1940, Fraenkel-Conrat, Meamber *et al.* (1940) employed cysteine to inactivate these contaminants and some further increase in growth-potency was achieved with ammonium sulfate fractionation or isoelectric precipitation (Marx, Simpson *et al.*, 1942b).

Adsorption methods have also been employed for the purification of growth hormone. Collip, Selye *et al.* (1933) have used calcium phosphate to adsorb the hormone, whereas Dingemanse and Freud (1935) employed activated carbon as the adsorbent and phenol as the eluent of the hormone. By these methods; however, they were unable to separate other active components from the growth hormone.

The isolation of the growth hormone in pure form has only recently been achieved in this laboratory (Li, Evans *et al.*, 1944, 1945). The success of the method depends on the following solubility characteristics of the hormone; (*a*) in the impure state, solutions of the growth hormone are precipitated after long-continued dialysis against water; (*b*) at pH 7.0, the hormone is soluble in 0.80 *M* but insoluble in 1.65 *M* $(NH_4)_2SO_4$; (*c*) the contaminating proteins are insoluble at pH 5.5 and pH 8.8 in the absence of electrolytes, whereas the growth hormone is soluble under these conditions; (*d*) at pH 4.0, the hormone is soluble in 0.10 *M* but insoluble in 1.0 *M* NaCl whereas a large part of the protein material is precipitated in 0.10 *M* NaCl pH 4.0 solution; (*e*) finally, in the absence of salt, the hormone possesses a minimum solubility at pH 6.8 which can be shown to be its isoelectric point.

b. The Procedure. The procedure employed for the isolation of the growth hormone from ox anterior pituitaries may be briefly described in the following steps:

(*a*) Acetone-dried powder of ox anterior pituitary. The freshly dissected anterior lobes of ox pituitaries were dried at −10°C. with acetone after they were ground into a fine mash. From 1 kg. of fresh anterior pituitary mash, approximately 250 g. of dried powder may be obtained. This material, if completely dried, can be stored indefinitely.

(*b*) Calcium hydroxide extract. The acetone-dried powder was extracted with a CaO solution of about pH 11.5 for 24 hours; the pH was then lowered to 8.7 by adding CO_2 gas. The insoluble material should settle easily within 24 or 48 hours. This step, like all other steps, was carried out at 2–3°C.

(*c*) The globulin fraction. The CaO extract was brought to 2.0 *M* $(NH_4)_2SO_4$ by the addition of solid $(NH_4)_2SO_4$. The resulting precipitate was suspended in water and dialyzed free of electrolytes. The insoluble material in the dialysis sac contains most of the growth-stimulating protein and may be called the "globulin fraction."

(*d*) NaCl fractionation. The "globulin fraction" was next suspended in water which was then brought to pH 4.0 with 1.0 *M* HCl; a saturated NaCl solution was then added until the salt concentration was 0.1 *M*. The 0.1 *M* NaCl precipitate, found to be devoid of growth activity, was removed by centrifugation. The supernatant was brought to 5.0 *M* with solid NaCl, and the precipitate formed was again suspended in water which was then brought to pH 4.0 as above. This NaCl fractionation was repeated twice.

(*e*) pH and $(NH_4)_2SO_4$ fractionation. The final 5.0 *M* NaCl precipitate was dissolved in water and dialyzed until salt-free. The dialyzed solution was adjusted to pH 5.7–5.8 and the precipitate formed was centrifuged off. The supernatant was then made alkaline and adjusted to pH 8.7–8.8, the precipitate again being removed by centrifugation. The clear fluid was then next brought to 1.65 *M* $(NH_4)_2SO_4$ at pH 7.0. The $(NH_4)_2SO_4$ precipitate was dissolved in water and dialyzed. The pH and $(NH_4)_2SO_4$ treatments were repeated twice.

(*f*) Isoelectric precipitation. The dialyzed solution of the final 1.65 *M* $(NH_4)_2SO_4$ precipitate was precipitated at pH 5.7–5.8 and pH 8.7–8.8 as above and finally at pH 6.8–6.9 in the absence of $(NH_4)_2SO_4$. This isoelectric precipitation was repeated twice. The final pH 6.8–6.9 precipitate is the growth hormone. In some cases, a pseudo-crystalline growth hormone can be obtained in this final isoelectric precipitation. Fig. 2 is a photomicrograph of the growth hormone particles.

From 1 kg. of fresh ox anterior pituitary, approximately 0.05 g. of the hormone was isolated. The hormone thus isolated was found to contain none, or only extremely low amounts, of biologically active contaminants. A total dose of 5 mg. in hypophysectomized female rats gives no histological evidence of the presence of adrenocorticotropic, thyrotropic, or gonadotropic hormones. No lactogenic activity was observed when 10 mg. was injected subcutaneously into month-old squabs (4 days). It was found that a 0.010 mg. daily dose of the hormone (9 injections) caused a 10 g. increase in the body weight of hypophysectomized female rats in a period of 10 days and that a 0.010 mg. daily dose for 4 days is sufficient to increase the width of the uncalcified portion of the proximal epiphyseal cartilage of the tibia 50% over that of the control.

c. Homogeneity Studies. The hormone isolated by the foregoing procedures has been examined as to its purity in electrophoretic, solubility, and diffusion experiments (Li, Evans *et al.*, 1945; Li, 1947). *Electrophoretic* studies were conducted in a Tiselius apparatus with the scanning method of Longsworth. The fraction obtained from each step in the purification was examined carefully as to its electrophoretic homogeneity. The increase of growth potency, the removal of biologically active contaminants and the decrease in the number of boundaries in electrophoresis were correlated so as to evaluate the usefulness of each purification step. In this way, one can ascertain the component in the electrophoresis patterns which represents the growth hormone. For instance, the material secured from step 5 possessed one main component together with two other components in smaller concentration, when subjected to electrophoretic examination in an acetate buffer of pH 4.0 and 0.10 ionic strength (see Fig. 3A). After isoelectric precipitation of this same fraction, the final product retained only the main component without loss of growth potency (see Fig. 3B). In other buffers at different pHs, the final isoelectric precipitate also behaved as a single substance. When a

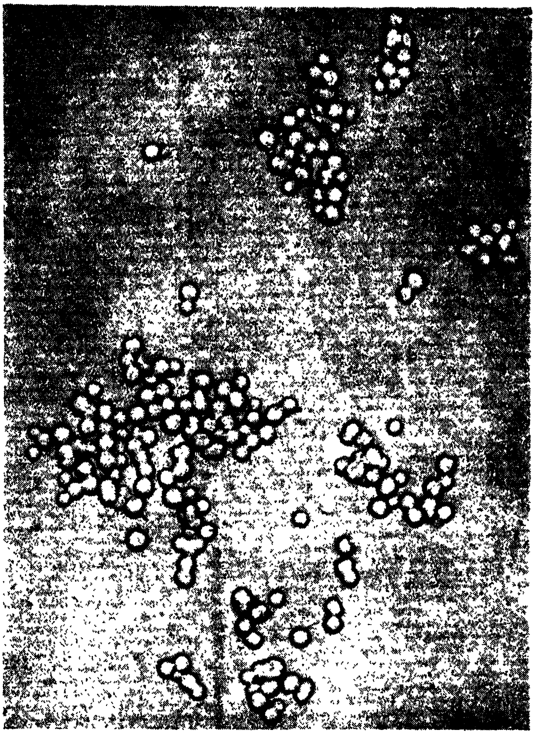


FIG. 2.—The growth hormone particles × 250.

1% growth hormone solution was electrolyzed in pH 4.0 acetate buffer at a potential gradient of about 6 volts/cm. for 120 minutes, separate recovery was made of the solution in the anode limb, in the cathode limb, and in the middle portion of the electrophoresis cell. After the nitrogen content was determined by micro Kjeldahl analysis, the separate fractions were assayed in hypophysectomized female rats for growth activity. It was found that there were no significant differences in the growth potency of these fractions. If the protein were not the hormone, marked decrease or increase of growth activity should be found in one of the fractions.

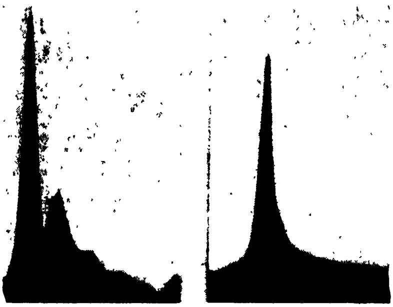


FIG. 3.—Electrophoresis patterns of (A) a growth hormone fraction before isoelectric precipitation and (B) the same fraction after the isoelectric precipitation in pH 4.0 acetate buffer of ionic strength 0.10, potential gradient about 6 volts/cc., 120 minutes electrolysis.

Diffusion experiments were carried out in both sintered glass disc and free diffusion techniques. A 0.5% growth hormone solution (pH 4.0 acetate buffer) was diffused through a sintered glass disc into 25 cc. of the same buffer which was replaced with fresh solution at definite time intervals. The amount of diffused nitrogen as determined by micro Kjeldahl analysis was practically constant, indicating uniformity in the molecular size of the protein. There was also no appreciable difference in the growth potency of the protein solutions before and after the diffusion experiment (Li, Evans *et al.*, 1945). Should the growth hormone be actually smaller in size than other protein present, it should to some extent have diffused from the main bulk of the protein and the remaining protein solution should have then exhibited some loss of growth activity. Another diffusion experiment (Li, 1947) was conducted in the electrophoresis cell at 2°C. From a series of diffusion patterns, a plot[2] of A/H_m against $\sqrt{t}$ gave a straight line relationship indicating the monodispersity of the preparation.

---

[2] A is the area under the diffusion curve, H_m the maximum height, and t the time in seconds.

The *solubility* behavior (Li, Evans *et al.*, 1945) of the growth hormone preparation has been studied in three different solvents. In each instance, the solubility of the hormone was constant after the appearance of the solid phase. The results, as shown in Fig. 4, indicate that the protein obeys the phase rule of Gibbs as a single pure component.

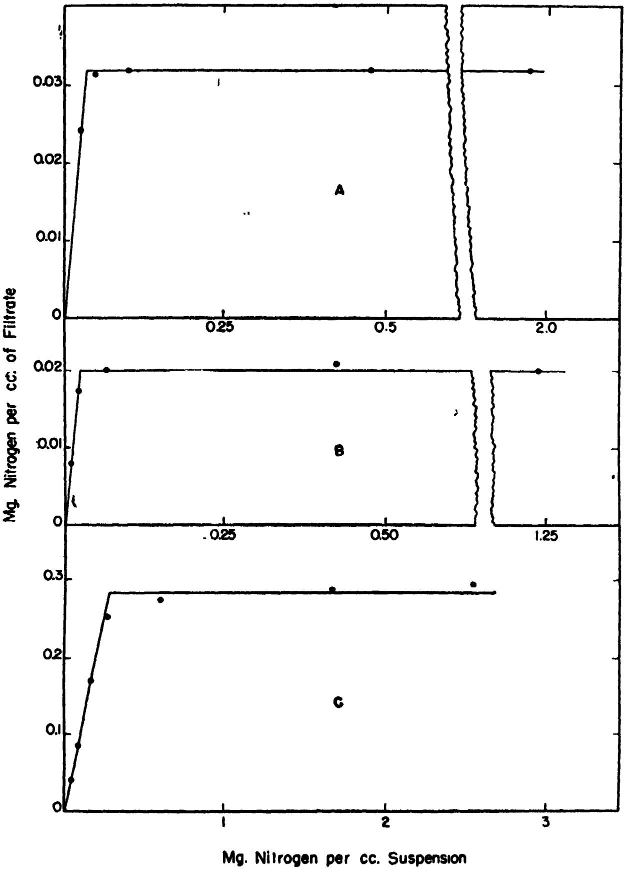


FIG. 4.—Solubility curves of the growth hormone in various solvents at 5°C. (A) Distilled water, pH 7.1; (B) 4.8 M NaCl in 0.07 M phosphate buffer, pH 5.7; (C) 3.8 M NaCl in 0.07 M phosphate buffer, pH 6.4.

3. Physical and Chemical Properties

a. Isoelectric Point and Molecular Weight. From electrophoretic mobility data (Li, Evans *et al.*, 1945) the isoelectric point of the growth hormone is estimated to be pH 6.85. This value is considerably higher than that found for lactogenic, adrenocorticotropic and sheep interstitial cell-stimulating hormones.

The osmotic pressure measurement (Li, Evans *et al.*, 1945) was employed for the determination of the molecular weight. When the protein is dissolved in 0.40 *M* or 0.80 *M* $(NH_4)_2SO_4$ solution at pH 6.64–7.00, the calculated molecular weight from the observed osmotic pressures is found to be 44,250. This value can also be derived from analytic, viscosity and diffusion data.

b. Analytic Data. Elementary analysis of the hormone gives the following results (Li, Evans *et al.*, 1945): Carbon, 46.35%; hydrogen, 7.07%; sulfur, 1.30%; nitrogen, 15.50% (Kjeldahl), 15.65% (Dumas). It contains neither phosphorus nor carbohydrate. The amino nitrogen and amide nitrogen are 0.76 and 1.2%, respectively.

The hormone contains 4.30% tyrosine, 0.92% tryptophan (Li, Evans *et al.*, 1945) and 13.4% glutamic acid (Lewis and Olcott, 1945). It has no cysteine. The sulfur in the protein is completely accounted for by 2.25% cystine and 3.06% methionine (Li, 1946a).

If one assumes that the hormone contains 2 molecules of tryptophan, 4 molecules of cystine, 9 molecules of methionine, 10 molecules of tyrosine, 40 molecules of glutamic acid and 18 atoms of sulfur, the average computed molecular weight (Li, 1947) for the growth hormone is found to be 43,575. This value is in good agreement with that obtained by the osmotic pressure method.

c. Diffusion and Viscosity. The diffusion constant, $D_{20^\circ,w}$, of the hormone (Li, 1947) as determined by the free diffusion technique is 7.15×10^{-7} cm.^{2}/sec. The viscosities of the hormone solutions (Li, 1947) as measured in a capillary viscometer give the constant for the specific viscosity as 7.64. This indicates that the ratio of the major to the minor axis of the protein is 6:1. Even if one assumes that the protein is hydrated to the extent of 30%, the growth hormone molecule still presents deviation from the spherical shape.

d. Stability. It has been generally believed that the material responsible for growth activity in crude extracts is a thermolabile substance (Evans, Meyer *et al.*, 1933; Shipley, 1942). Using the isolated hormone it was found (Li, Evans *et al.*, 1945) that the hormone activity was completely lost at pH 4.0, 7.5, and 8.9 at 100°C. for 10 minutes. When an intermediate temperature was applied for 1 hour, it was observed that the hormonal solution (0.02% at pH 7.0) remained perfectly clear up to 60°C., turbidity began at 70°C. and flocculent precipitation occurred at 80°C. On the other hand, the growth potency was not significantly reduced at 60°C. but it was destroyed at 70°C. and 80°C. This would indicate that the hormonal activity is identified with the protein.

It was further found that the hormone is more stable in alkaline than in acid solutions and that the growth activity is not destroyed by urea denaturation. Both trypsin and pepsin digestions abolish the growth potency.

When the growth hormone was treated with nitrous acid or ketene, the product exhibited no biological activity. It was concluded from these experiments that free amino groups are essential for the growth-promoting action (Li, 1946b).

4. *Biological Properties*

a. Body Growth. As the name of the hormone implies, the first observed effect of the hormone in either normal or hypophysectomized animals is the gain in body weight. The name growth hormone further implies that it selectively increases the body growth independently of its effect on other endocrine organs, some of which are known to be affected by the pituitary. It was found that growth hormone extracts were equally active in adrenalectomized animals (Simpson, Marx *et al.*, 1944) and in those which were completely thyroidectomized (Scow and Marx, 1945) and that the gonads are not required for the action of the growth hormone (van Wagenen, 1928; Evans and Simpson, 1931). However, the responsiveness of adrenalectomized-thyroidectomized-gonadectomized-hypophysectomized animals to growth hormone is yet to be investigated. We can say with certainty that the pure growth hormone promotes body growth in hypophysectomized rats without stimulation of the gonads, adrenals or thyroids.

There has been some discussion as to the ability of the growth hormone to cause *continuous* growth in normal or in hypophysectomized rats. To sum up the situation Long (1943) has said: "In the first experiments reported by Evans and Long the rats were injected daily for as long as 8–13 months with a crude alkaline extract and, although growth was not as rapid in the late period as at first, nevertheless it was continuous throughout the period of injections. Later attempts to repeat this experiment, even in the same laboratory, showed that, after an initial period of brisk growth, the animals became refractory to the extract and even lost some of the weight they had gained. The same results were also obtained in hypophysectomized animals but, even more discouraging, was the fact that partial purification of the extract did not correct this decreased responsiveness." Gaebler also, in studies with dogs more than 10 years ago, found that repeated injections of a growth preparation (Parke, Davis) resulted in the simultaneous disappearance of all responses, but that these responses returned after a 2-months' interval as shown in Fig. 5. Since practically all preceding experiments have been carried out with only partially purified growth-promoting extracts, it is naturally difficult to decide as to whether such results were due to the growth hormone itself or to other contaminating proteins.

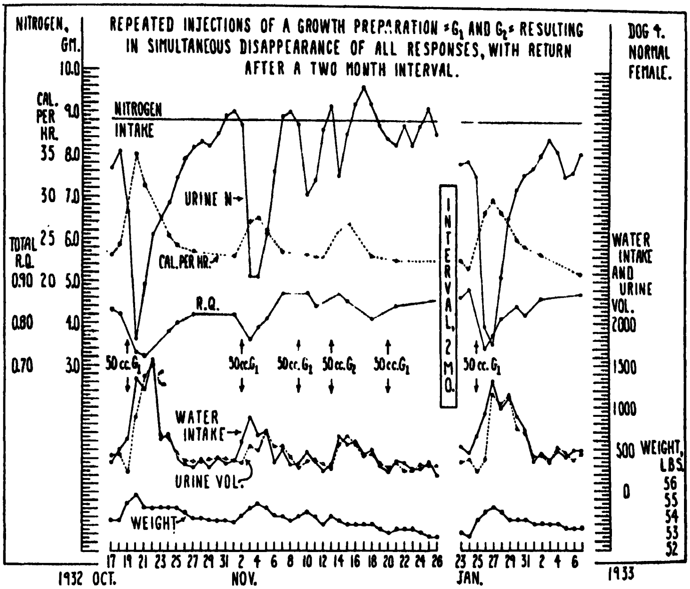


FIG. 5.—Responses of a dog to a growth preparation (Parke, Davis) over 4 months period. (Gaebler, 1946.)

Evans, Simpson, and Li (1946) have recently injected the isolated hormone into normal adult female rats for 435 days; daily dose increased gradually from 0.4 mg. to 2.0 mg. Growth continued during whole period, the experiment being terminated due to the advanced age of the animals. The greatest weight attained was 662 g. The range of final weights in the experimental and control groups did not overlap; the smallest experimental rat weighed 410 g., the largest control 353 g. The average gain of the 8 experimental rats was 293 g., that of the controls 57 g. The average body length at autopsy of the experimental rats was 45.5 cm., that of the controls, 40.9 cm. Liver, kidneys, heart, stomach and intestine increased in weight in proportion to body weight. The thymus was not hypertrophied as occurs upon acute administration of growth hormone. The other endocrine organs were not increased proportionally to body weight, as might be anticipated from the absence of the specific hormonal stimulants in the growth hormone treated. Fig. 6 presents a photograph of one of these giant rats as obtained by growth hormone injections in comparison with its control.

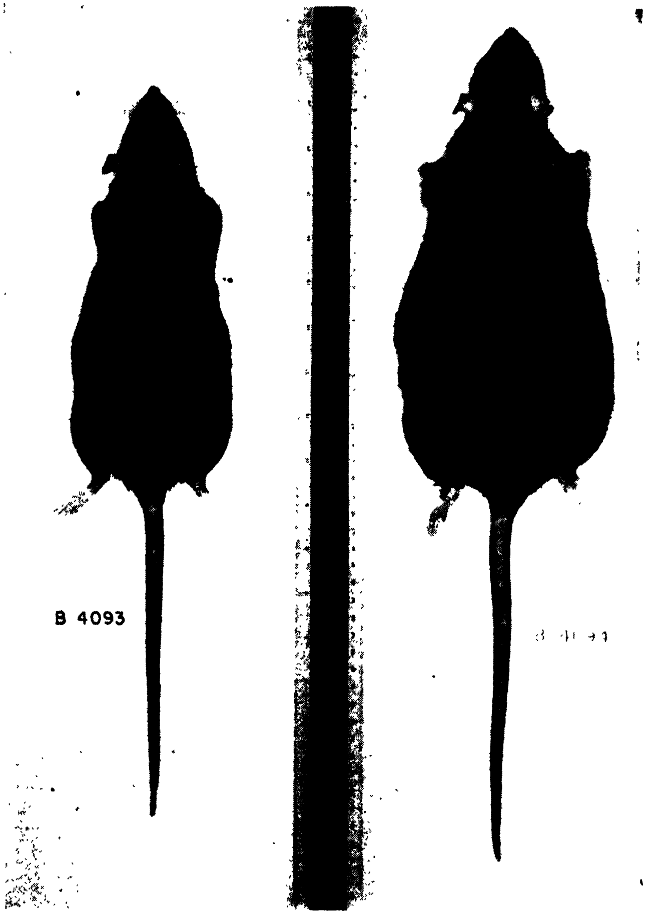


FIG. 6.—Typical photograph of a normal plateau female rat receiving growth hormone for 432 days as compared with the control. For the dose of hormone used, see the text. B 4093, Control. B 4094, Injected.

A similar experiment with hypophysectomized female rats, 26–28 days of age and 12–14 days postoperative, also indicates that the pure growth hormone induces continuous growth in such animals and there is no sign of refractoriness with over 400 days of injection. The daily dose required in this experiment was much less than that for normal rats; it changed from an initial dose of 0.10 mg. to 0.20 mg. in the later period of the experiment. Though the experiments with hypophysectomized animals are still in progress, we wish to present the growth rate of an injected rat and its control in Fig. 7. It can be seen that the body weight of the hypophysectomized rat gained 397 g. in 420 days whereas the control gained only 32 g.

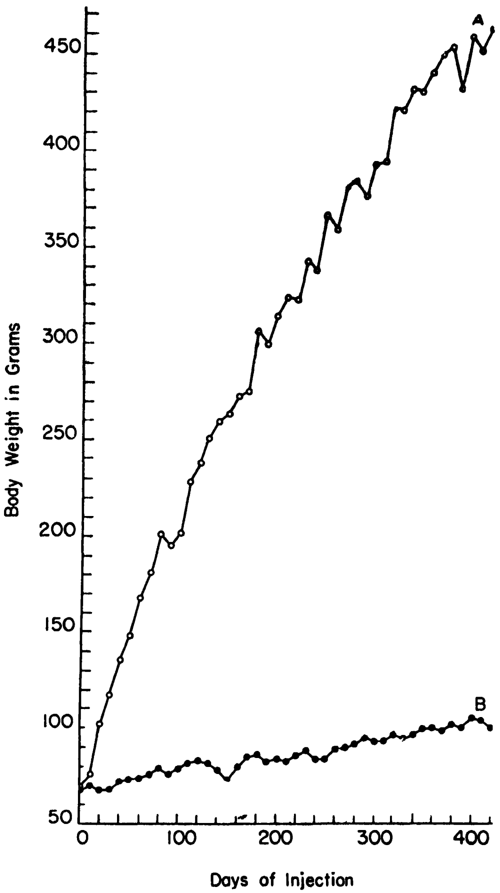


FIG. 7.—Growth curves of hypophysectomized female rats. (A) The experimental animal received 0.10 mg. daily growth hormone the first 140 days and then increased the daily dose to 0.20 mg.; no injections on Sunday. (B) The control received no injections.

b. Nitrogen Retention. Since true growth is generally interpreted as the accumulation of proteins, it is reasonable to expect, from the foregoing discussion, that an important function of the growth hormone is to retain nitrogen which, in turn, increases the protein content of the body tissues. Earlier reports (Teel and Watkins, 1929; Gaebler, 1933;

Harrison and Long, 1940) have already shown that growth-promoting pituitary extracts cause a reduction in both the blood non-protein nitrogen and in the urinary nitrogen. Later experiments (Marx, Magy *et al.*, 1942; Fraenkel-Conrat *et al.*, 1942) with partially purified growth hormone have confirmed these conclusions.

The changes in the body composition of animals after treatment with pituitary extracts causing accelerated growth have been studied by several workers (Downs, 1930; Wadehn, 1932; Bierring and Nielsen, 1932; Lee and Schaffer, 1934). The results of these investigations indicate that the weight gain of treated animals is due to increase in protein and water content and decrease in the fat constituents of the body. Kleiber and Cole (1939), however, did not find significant differences in the ash, fat or protein content of injected and control rats. The same investigators have also studied the metabolic rate in rats made gigantic by chronic administrations of growth-promoting extract. Results showed that the metabolic rate per unit body weight was less in injected animals than in the controls and that the metabolic rate *in vitro* per unit dry weight of the diaphragm of the giant rats was also lower than that of the controls. These changes in the metabolic rate make their reported lack of alteration in the water or protein content of the animals all the more surprising.

Although all the experiments cited were performed with crude or partially purified preparations, it appears likely that the growth-promoting activity of pituitary extracts is accompanied by the reduction of urinary nitrogen and increase of body proteins. These changes may be interpreted as being caused by either an increase of protein anabolism, a decrease of protein catabolism, or both. From the experiments of Fraenkel-Conrat, Simpson *et al.* (1943) on the effect of purified growth hormone on liver arginase content, decrease in the latter indicates that the purified hormone has indeed inhibited protein catabolism.

Recent studies with the pure growth hormone have shown that the hormone reduces the urinary nitrogen markedly in various conditions. In some cases, the disappearance of urinary nitrogen corresponds almost quantitatively to the gain in body weight (Gordan, Li *et al.*, 1946a). In normal rats with constant diet containing 24% casein, injections of growth hormone induce a significant lowering of the urinary nitrogen within 24 hours. It will be seen that rats made diabetic by alloxan also retain nitrogen after growth hormone treatment. The nitrogen-retaining effect of the growth hormone has also been demonstrated in rats with bilateral fracture of the femur (Bennett, Applegarth *et al.*, 1946). It was found that the normal and injured rats given growth hormone showed approximately the same decrease in nitrogen excretion when compared with their respective controls.

c. Stimulation of Epiphyseal Cartilage. It has already been mentioned that the growth hormone brings about a specific stimulation of the epiphyseal cartilages in hypophysectomized rats. The first change observed in these animals after hypophysectomy (Becks, Simpson *et al.*, 1945) is a marked thinning of the epiphyseal cartilage plate, due to decrease in number, and particularly in size, of cells. Recently Becks, Simpson *et al.* (1946) have shown that administration of growth hormone to hypophysectomized rats, even after postoperative intervals of a year or longer, was able to reawaken chondrogenic and osteogenic processes in the epiphyseal cartilage of the tibia to an extent comparable to that seen in normal, young, growing rats. Fig. 8 presents central sagittal sections of the proximal epiphyseal cartilages of a tibia of a growth hormone-treated hypophysectomized rat in comparison with the epiphyseal cartilage of its control. It may be noted that the animal receiving growth hormone had enlarged cartilage cells in the juxtamedullary zone and a wide zone of newly-formed, delicate trabecular bone. There are numerous and active osteoblasts at the margin of the cartilage and along the surfaces of the trabeculae. There could, therefore, be little doubt that the growth hormone has a direct influence on bone growth.

The relation of phosphatase to bone regeneration has been demonstrated by Wilkens and Regen (1935). In the growth of normal rats, a rise in plasma phosphatase activity from birth to maturity was observed by Weil (1941). One could, therefore, expect that growth hormone would increase the phosphatase content in the tissues of normal or hypophysectomized rats. Results yet to be published indicate that the plasma phosphatase content of hypophysectomized male rats is more than double the content in controls, after treatment with as small a dose as 0.05 mg. daily for 14 days. This increment in the phosphatase content of the blood after the administration of the hormone suggests some relation of the enzyme to the process of bone formation. Further studies on other biochemical changes in the bone and blood are in progress.

d. Diabetogenicity. It is well known that hypophysectomized animals exhibit hypersensitivity to insulin and a respiratory quotient higher than normal. Fasting in these animals develops hypoglycemic convulsions. Russell (1938, 1942) has shown that anterior pituitary extracts decrease the rate of fall of blood sugar levels in eviscerated rats and increase the carbohydrates in hypophysectomized rats. These different phenomena have established a "diabetogenic" action of the anterior pituitary or its extracts. There is ample evidence that not all effects of anterior pituitary extracts on carbohydrate metabolism are mediated through the adrenal cortex. For instance, Houssay and Leloir (1935) found that the dia-

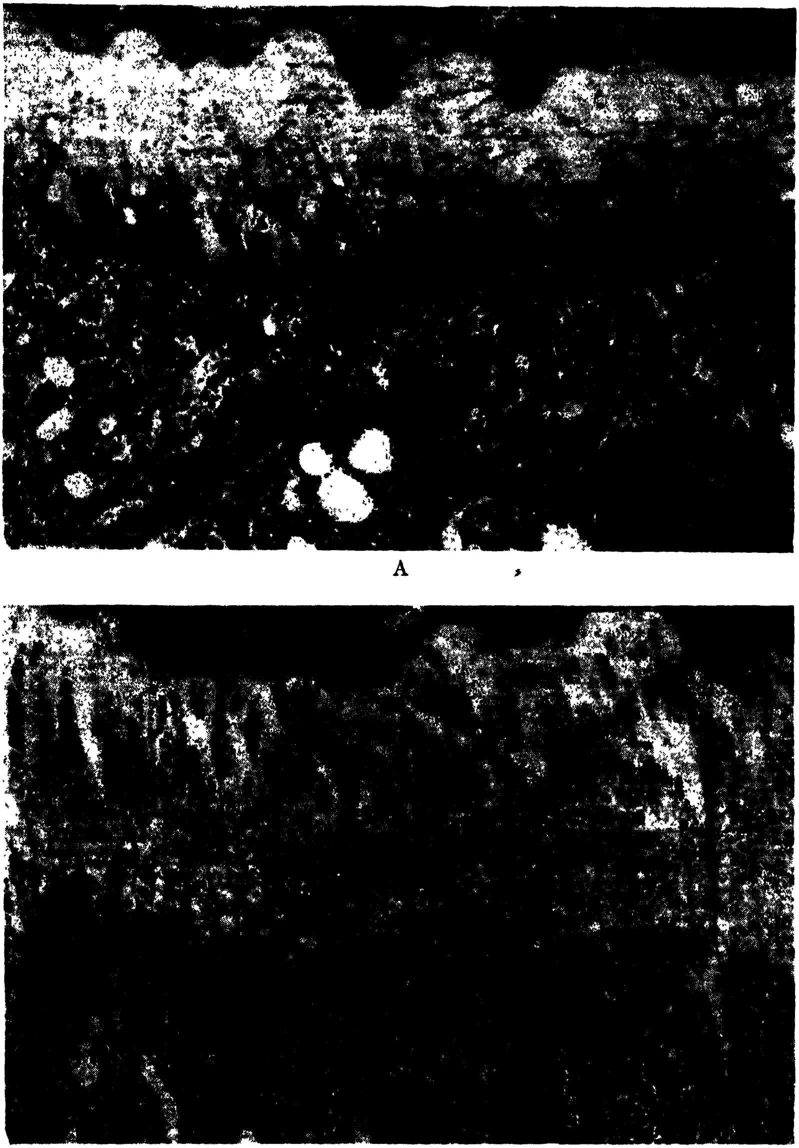


FIG. 8.—Central sagittal sections of proximal epiphyseal cartilage of the tibia of hypophysectomized female rats. Haematoxylin and eosin stain, 8 to 10 μ, × 60. A. Uninjected control, 387 days after hypophysectomy at 27 days of age. B. Growth hormone, 0.20 mg. daily for 39 days, beginning 285 days after hypophysectomy at 28 days of age.

betogenic activity of the anterior pituitary can also be observed in adrenalectomized animals.

The experiments of Shipley and Long (1938) and of Young (1939, 1945) indicated that the diabetogenic factor in anterior pituitary extracts may be identical with the growth-promoting hormone. Some investigators believe that the growth-promoting action of the anterior pituitary requires the presence of the pancreas. Mirsky (1939) showed that pituitary extracts rich in growth-promoting activity increase the rate of accumulation of non-protein nitrogen in the blood of nephrectomized-depancreatized dogs, but the reverse result was obtained in nephrectomized dogs with pancreas intact.

In rats diabetic from alloxan and maintained on a constant food intake, a significant nitrogen retention and gain in body weight were observed upon the injection of the pure growth hormone (Bennett and Li, 1946). With high doses of the hormone, half the group of animals showed an increase in glycosuria, whereas at the low dose level this was not elicited. Similar results were obtained with alloxan-diabetic rats receiving concurrent definite levels of insulin.

III. ADRENOCORTICOTROPIC HORMONE

1. *Methods of Assay*

a. Historical. It is well known that the pituitary influences the size and function of the adrenal cortex. The first evidence for the occurrence of an adrenocorticotropic substance in pituitary extracts was given by Smith (1930), and it appears that, although hypophysectomy decreases the adrenal cortex, it is without effect on the medulla. The isolation of the adrenocorticotropic hormone in pure form has been achieved independently by two laboratories (Li, Simpson *et al.*, 1942b, 1943; Sayers, White *et al.*, 1943).

The adrenal weight in rats or chicks has been proposed for the estimation of adrenocorticotropic potency. Collip and coworkers (1933) suggested the removal of one adrenal from the hypophysectomized rat as the control, the weight of which was then compared with the weight of the remaining adrenal after the animal had received an adrenocorticotropic extract. Moon (1937a) employed the 21-day old male rat as the experimental animal; he injected intraperitoneally once daily for 3 days and the weight of the adrenals on the 4th day was compared with that of uninjected controls. Later, Moon (1940) reported the use of 4-day old suckling rats; the method seems to be more sensitive but it has a disadvantage in that it is difficult to inject crude extracts or those containing toxic substances into such young animals. Bates, Riddle *et al.* (1940) used the increment in adrenal weights in 2-day old chicks for assaying adrenocorticotropic preparations. The method was found unsatisfactory in our hands; perhaps due to the different strain of chicks employed.

Recently the repair (Simpson, Li *et al.*, 1943) or maintenance (Simpson, Li *et al.*, 1943; Astwood and Tyslowitz, 1942) of the adrenals of hypophysectomized rats has been used for the standardization of the adrenocorticotropic hormone. Both methods are sensitive and highly specific.

b. The Repair Test. The histological change in the adrenal cortex of rats after hypophysectomy is characterized by the appearance of a specific sudanophobe zone (Reiss, Balin *et al.*, 1936). This is due mainly to the change in distribution of lipids; the lipids also become large and more irregular in size in the hypophysectomized animals. The repair test is thus based on the ability of adrenocorticotropic hormone to amend these changes. Female rats, 26–28 days of age were hypophysectomized and the adrenals allowed to regress for 14 days, by which time injections were instituted and continued for 4 days, once daily intraperitoneally, followed by autopsy 96 hours after the first injection. The adrenals were fixed in formol, cut as frozen sections, and stained with Sudan Orange. The slightest observable effect of pure adrenocorticotropic hormone caused a beginning redistribution of the lipids. This minimal effective dose lies somewhere between 0.01 and 0.025 mg. Fig. 9 shows these changes with different dose levels of the hormone.

c. The Maintenance Test. The second test is based on weight maintenance of the adrenals by the institution of injections immediately after hypophysectomy. Male rats 40 days old were hypophysectomized and injected intraperitoneally, daily (except Sunday) from the day of operation for 15 days (14 injections). The adrenal weight of uninjected hypophysectomized animals regressed during this period from 26 mg. to a constant weight of 12 mg. The amount of pure hormone which maintains the adrenal at 26 mg. is about 0.2 mg. daily dose. It must be emphasized that this value was obtained from *single* daily injections. It is known that multiple daily injections of adrenocorticotropic hormone produce a better adrenal response from any given dose. White (1945) has shown that the sensitivity of the method is influenced by the strain of rats employed; he further showed that if the adrenal size is expressed per 100 g. of body weight, assays in different laboratories can be satisfactorily compared.

2. Methods of Isolation

Methods for isolating adrenocorticotropic hormone utilized acid-acetone extract of fresh glands as the starting material. This acid-acetone extract, first suggested by Lyons (1937), is particularly rich in lactogenic and adrenocorticotropic potency and low in thyrotropic, gonadotropic and growth activities. In our experience these facts are extremely useful in the isolation of the first mentioned hormones from pituitary tissue. We must admit, however, that industrially employed

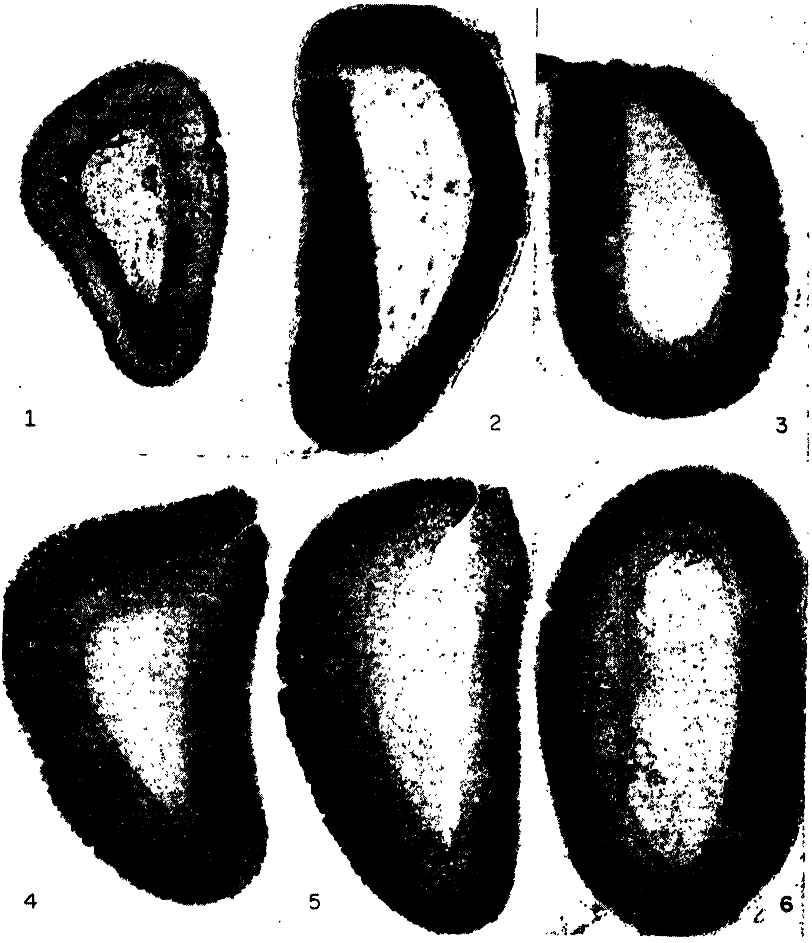


FIG. 9.—Repair of the adrenal cortex of hypophysectomized rats by adrenocorticotropic hormone. No. 1, control; No. 2, total dose 0.025 mg.; No. 3, total dose 0.05 mg.; No. 4, total dose 0.10 mg.; No. 5, total dose 0.20 mg.; No. 6, total dose 1.0 mg.

this would be an expensive process, for it injures or destroys the three last-mentioned hormones. Yet, when our object is merely to isolate the adrenocorticotropic hormone and study its physicochemical properties, we can neglect other considerations. After the characteristics of each of the hypophyseal hormones are known, procedures for the isolation of all of them from a single starting extract could conceivably be worked out.

a. Swine Hormone. Sayers, White and Long (1943) used fresh swine pituitaries as their original material and proceeded to obtain the hormone by a method based on the principle that both adrenocorticotropic hormone and its main contaminant (lactogenic hormone) have their lowest solubility at their isoelectric point. The product thus obtained was found to behave as a homogeneous material in electrophoretic and ultracentrifugal experiments.[3] Their method may be briefly outlined in the following steps:

(*a*) One kg. of whole swine glands is extracted with acid-acetone as described by Lyons (1937). The extractable material is precipitated by increasing the acetone concentration to 92%. The precipitate is extracted 4 times with 50 cc. of water. The so-called "crude prolactin" fraction is obtained by adding acetone to the combined aqueous extracts to a concentration of 92%.

(*b*) The "crude prolactin" is dissolved in water at pH 9.0. The precipitates formed by lowering the pH to 8.0, 6.6 and 5.4 successively are removed by centrifugation. The supernatant at pH 5.4 is brought to approximately 0.07 saturated ammonium sulfate. After removal of the small amount of precipitate formed, 4 volumes of acetone are added.

(*c*) The precipitate is dissolved in water and the solution is mixed with one-half its volume of concentrated ammonium hydroxide. After the ammoniacal solution is allowed to stand at room temperature for 7 hours, acetone is added to 90%.

(*d*) The precipitate is dissolved in water and dialyzed until salt-free; any precipitate formed during dialysis is discarded. The solution is adjusted to pH 5.4 and centrifuged free of the precipitate. The pH is next lowered to 4.7; the precipitate is adrenocorticotropic hormone.

The hormone isolated has been tested in hypophysectomized male rats and pigeons as to its freedom from growth, thyrotropic, gonadotropic and lactogenic activities. It may be mentioned that the absence of alterations in the *weight* of the thyroid or testes were used by Sayers, White *et al.* as reliable indications of the absence of thyrotropic and gonadotropic contaminants. It is, however, well known that the weight changes in thyroid or testes of hypophysectomized animals after injecting thyrotropic or interstitial cell-stimulating hormones are comparatively slight. In our experience, histologic studies should be used for indications of the absence or presence of these two contaminants in a purified preparation.

b. Sheep Hormone. Li, Simpson and Evans (1942b, 1943) used fresh whole sheep glands as the starting material for obtaining the adrenocorticotropic hormone. The procedure is outlined as follows:

---

[3] The purity of the Yale preparation has not yet been examined from the standpoint of solubility studies.

(*a*) Sheep pituitaries are ground and extracted with acidified acetone. The extract is precipitated in 90% acetone and dried.

(*b*) The precipitate is extracted with 0.10 *M* Na_2HPO_4; the extract is next precipitated in 2.0 *M* $(NH_4)_2SO_4$. The precipitate is dissolved in water and dialyzed until salt-free.

(*c*) The dialyzed solution is brought to 0.54 *M* NaCl at pH 3.0, and centrifuged. The supernatant is again brought to 2.0 *M* $(NH_4)_2SO_4$.

(*d*) The precipitate is dissolved in water and the solution has half its volume of concentrated NH_4OH added to it. The ammoniacal solution is allowed to stand at room temperature for 4 hours.

(*e*) The solution is then brought to 90% acetone. The precipitate formed is dissolved in water and dialyzed against pH 7.5 phosphate buffer.

(*f*) Saturated $(NH_4)_2SO_4$ is added to the dialyzed solution until a 1.65 *M* solution results. The precipitate is dissolved in water and dialyzed again against pH 7.5 phosphate buffer.

(*g*) The dialyzed solution is put into a boiling water bath for 2 hours. After the heat treatment, $(NH_4)_2SO_4$ fractionation is repeated.

(*h*) The final 1.65 *M* $(NH_4)_2SO_4$ precipitate is dissolved in water and brought to 0.54 *M* NaCl and then to 1.36 *M* at pH 3.0. The precipitate, coming down between 0.54 *M* and 1.35 *M* NaCl, is adrenocorticotropic hormone.

It was found that from 1 kg. of whole sheep glands approximately 70 mg. of the hormone could be isolated. When assayed in hypophysectomized male or female rats, the hormone was shown to be freed from follicle-stimulating, interstitial cell-stimulating and thyrotropic contaminants as judged by histologic changes. It was also evident that the hormone was free from lactogenic and growth activities when it was assayed in pigeons and adrenalectomized rats. Pressor substances from the posterior lobe of the pituitary were also found to be absent. Thus, it can be stated that the sheep hormone is completely free from other biologically active components save for its own specific adrenocorticotropic action.

The physicochemical purity of sheep hormone has been examined by electrophoresis, ultracentrifuge, diffusion and solubility studies. When the hormone solution is electrolyzed in a Tiselius electrophoresis apparatus at 7 different pHs, it behaves as a single protein. Solubility experiments in one solvent give a curve indicative of a single component.[4]

---

[4] The protein is found to be homogeneous in ultracentrifugal experiments, but the results from one diffusion experiment carried out in a Lamm cell gave evidence of some inhomogeneity (Burtner, 1943).

3. *Physical and Chemical Properties*

a. Isoelectric Point. The isoelectric point of adrenocorticotropic hormone has been thought for some time to lie at pH 6.0–6.5 (Lyons, 1937). When the hormone was isolated in pure form, electrophoretic data revealed the fact that the isoelectric point of the sheep hormone is between pH 4.65 and 4.70 (Li, Simpson *et al.*, 1943) while this point in the case of the swine hormone lay between pH 4.7 and pH 4.8 (Sayers, White *et al.*, 1943). Although these two proteins have thus almost identical isoelectric points, electrophoretic studies at other pHs may reveal differences with respect to electrochemical properties. Unfortunately, the data given by Sayers *et al.* (1943) do not specify the ionic strength of the buffers used; and it is hence not possible to make suitable comparisons of the electrophoretic behavior of the two proteins.

b. Molecular Kinetic Data. Burtner (1943) obtained sedimentation and diffusion constants for the sheep adrenocorticotropic hormone isolated by Li *et al.* to be: $S_{20} = 2.08$ S, $D_{20} = 10.4 \times 10^{-7}$ cm.^{2}/sec. If the partial specific volume is assigned as 0.75, the molecular weight is computed to be 20,000; the frictional ratio, f/f_0, becomes 1.1 which gives as an approximate value 3:1 for the ratio of major to minor axis of the assumed unhydrated ellipsoidal molecule.

Sayers, White *et al.* (1943) gave the sedimentation constant of their swine hormone, $S_{20} = 2.04$ to 2.11S. They reported no diffusion data but an estimated molecular weight of approximately 20,000.

c. Some Analytical Data. The elementary analysis of adrenocorticotropic hormone isolated from sheep glands shows it to contain 46.3% carbon, 5.89% hydrogen, 15.65% nitrogen and 2.30% sulfur. There is no carbohydrate, phosphorus, or cysteine in the hormone. The hormone contains 1.93% methionine and 7.19% cystine (Li, 1946a); the content of these sulfur-containing amino acids fully accounts for the amount of sulfur in the protein.

d. Solubility. The adrenocorticotropic hormone is very soluble in water and is only partly precipitated at its isoelectric point. In 2.5% trichloroacetic acid solution, it is almost completely precipitated (Li, Simpson *et al.*, 1943). The hormone is readily precipitated from dilute solution by 20% sulfosalicylic acid and by 5% lead acetate solution (Sayers *et al.*, 1943). Bates, Riddle *et al.* (1940) reported that their adrenocorticotropic principle had an appreciable solubility in 60–70% ethanol or acetone, even in the isoelectric region.

e. Stability. One of the remarkable properties of adrenocorticotropic hormone is its resistance to heat treatment (Li, Simpson *et al.*, 1943). When a hormone solution in a buffer of pH 7.5 is put in a boiling water bath for 120 minutes or longer, no loss of adrenocorticotropic activity is observed. In 0.10 *M* HCl, 0.2% hormone solution retains its biological potency after being kept at 100°C. for 60 minutes, but when a hormone solution of the same concentration in 0.10 *M* NaOH is heated to 100°C. for 30 minutes, hormonal activity is almost completely destroyed. Experiments conducted at 60°C. for 60 minutes in the case of a 0.2% adrenocorticotropic hormone solution at pH 10.8, showed no significant loss in potency (Li, 1946b). The heat resistance of the adrenocorticotropic activity in an acid solution has also been observed in the case of a partially purified preparation by Noble and Collip (1941).

f. Effect of Various Agents. The reactions of adrenocorticotropic hormone with ketene, nitrous acid, formaldehyde and iodine have been studied (Li, Simpson *et al.*, 1946) and the results suggest that both the free amino and tyrosine groups are essential for the hormonal action. The hormone remained active after certain pepsin digestions but was destroyed by the enzymatic action of trypsin (Li, Evans *et al.*, 1945).

4. *Biological Properties*

a. Inhibition of Growth. Using partially purified adrenocorticotropic extract, Moon (1937b) found that it caused a retardation of the somatic growth of young castrated male rats. Recently, employing a pure adrenocorticotropic hormone we have observed a similar effect on the growth of normal as well as of gonadectomized male rats (Evans, Simpson *et al.*, 1943). It was further shown that this effect disappeared if adrenalectomized rats were used. These results are in harmony with the findings of Ingle, Higgins *et al.* (1938) and of Wells and Kendall (1940) who have shown that certain adrenocortical substances themselves adversely affect growth, and the adrenocorticotropic hormone is thus shown to increase the output of these substances on the part of its target organ.

When adrenocorticotropic hormone is injected simultaneously with the growth hormone in hypophysectomized rats a counteraction exists between these two substances (Marx, Simpson *et al.*, 1943). It was found that the growth activity is antagonized by the action of adrenocorticotropic hormone. This antagonism may be demonstrated both by the measurement of body weight increase and by the degree of proliferation of the proximal epiphyseal cartilage of the tibia.

The inhibiting property of adrenocorticotropic hormone is always clear when one investigates the osseous system. The treatment of normal rats with the hormone resulted in a retardation in both chondrogenesis and osteogenesis in the region of the proximal epiphysis of the tibia (Becks, Simpson *et al.*, 1944a). Comparisons have been made of the proximal epiphyseal regions of the tibia of hypophysectomized rats when injected with adrenocorticotropic hormone, with growth hormone or with the combination (Becks, Simpson *et. al.*, 1944b). The adrenocorticotropic hormone given alone can scarcely further modify the inactive condition of the epiphysis of hypophysectomized animals, whereas growth hormone always causes a resumption of activity here. When adrenocorticotropic hormone is administered concordantly with a known effective dose of growth hormone the following effects were observed:—

(*a*) The proximal epiphyseal cartilage of the tibia was greatly decreased in width when compared with the width always produced by this dosage with growth hormone when the latter is administered alone.

(*b*) Endochondral bone formation was significantly retarded.

(*c*) Osteoblasts as well as osteoclastic activity was greatly decreased, perhaps accounting for the irregular arrangement of bony trabeculae.

(*d*) The cartilage columns in the erosion zone were also more irregular. Fig. 10 illustrates the typical results of these experiments.

From the foregoing, it is apparent that the adrenocorticotropic hormone may be regarded as a specific growth-inhibiting substance. A complete explanation of the mechanism of its action is not yet in our hands, but there is no doubt that adrenocorticotropic hormone adversely influences certain metabolic reactions that promote growth. For example, Gordan, Li *et al.* (1946b) have found that adrenocorticotropic hormone causes an increase in urinary nitrogen excretion with proportionate loss of body weight in the normal rat and that the effect is manifest on the second day of hormone administration and persists for 24 hours after injection is stopped. Fraenkel-Conrat, Simpson *et al.* (1943) showed that liver arginase is increased by the administration of adrenocorticotropic hormone as well as by certain adrenal cortical substances, whereas growth hormone induces a decrease of the enzyme activity. Recently we were able to show that adrenocorticotropic hormone reduces the alkaline phosphatase content in the plasma of both hypophysectomized and normal rats (Li, Kalman *et al.*, 1946) and that the effect is neutralized by growth hormone injections (Li, 1946b).

b. Effect on the Gonads of Male Rats. It now appears certain that the adrenal cortex functions as an extragonadal source of androgens (Parkes, 1945). Holder (1937) observed a positive response of the accessory organs in castrated male guinea pigs receiving beef adrenal implants or extracts. Leonard (1944) maintained the size and function of the adrenal cortex of hypophysectomized rats by the administration of some androgenic steroids. The fact that the adrenal cortex contains androgenic substances is also evident from the work of Reichstein (1936, 1938) who has isolated two steroids with adrenogenic properties from beef adrenals. It appears, therefore, of interest to investigate whether any androgenic

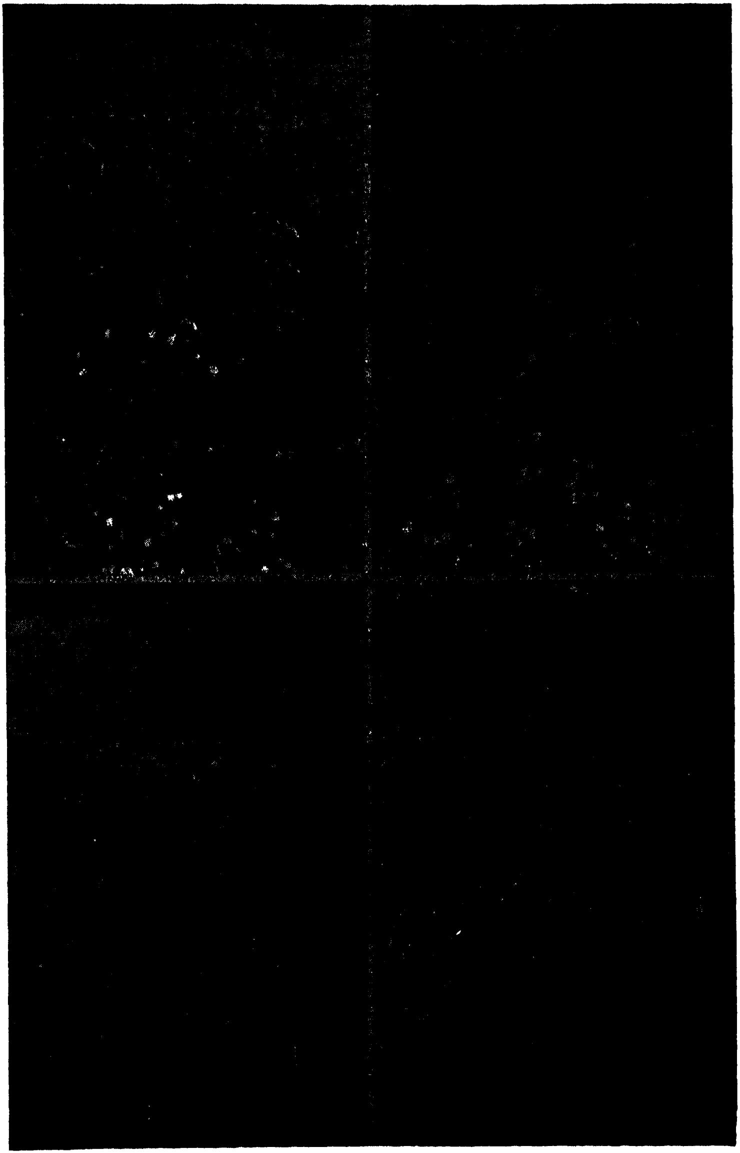


FIG. 10.—Proximal epiphyseal cartilage of right tibia of rat, hypophysectomized 26 days; 56 days of age at autopsy. Central sagittal section, Hematoxylin and eosin, 8 μ, × 24. No. 1, Untreated control. No. 2, Injection with 0.1 mg. adrenocorticotropic hormone daily for 15 days, total dose 1.3 mg. No. 3, Injection with 0.1 mg. growth hormone daily, total dose 1.3 mg. No. 4, Injection with growth hormone and adrenocorticotropic hormone, doses as above.

activity is exercised by the adrenals when under stimulation by the adrenocorticotropic hormone. In this connection, we may recall the results of Davidson and Moon (1936). They found that a pituitary extract rich in adrenocorticotropic activity caused enlargement of the seminal vesicles and prostates of castrated rats. We have investigated this problem with the pure hormone and the results may be summarized as follows:—

(*a*) Administration of pure adrenocorticotropic hormone in 1 mg. daily doses for 30 days to normal 26-day old male rats depressed the growth rate of the testes, seminal vesicles, prostate and Cowper's glands. The seminal vesicles were the most markedly affected. Microscopic examination of the testes revealed that development had been retarded by the treatment, the tubules were smaller, and fewer of them contained mature spermatozoa than in the normal controls. There was, however, no evidence of tubular injury and desquamation of germinal elements.

(*b*) The reproductive organs of adult male rats (89 days of age) were not affected by treatment with the hormone at 1 mg. daily dose for 15 days.

(*c*) Administration of pure adrenocorticotropic hormone at various dose levels to hypophysectomized 40–43-day old male rats for 15 days did not cause maintenance of the weights of the testes, seminal vesicles or prostate.

(*d*) When a daily dose ranging from 1 to 4 mg. was given for 15 or 25 days to male rats castrated when from 30–40 days of age and a fortnight allowed for regression of the accessories there was no change in the weights of the seminal vesicles or prostates. These accessory organs in hypophysectomized-gonadectomized rats have also proven equally unresponsive to large doses of the pure hormone.

c. *Effect on Carbohydrate Metabolism.* It is well known that anterior pituitary extracts are able to maintain carbohydrate stores in fasted hypophysectomized animals. Herring and Evans (1943) show that adrenocorticotropic hormone does not maintain normal muscle glycogen levels when it is given only during the fasting period. However, when adrenocorticotropic hormone is given for longer periods of time so that adrenal cortical hypertrophy is produced, maintenance of muscle glycogen and hyperglycemia is found during fasting. These results are similar to those produced by adrenal cortical steroids in large doses (Long and Katzin, 1938) and are best interpreted as being secondary to the production of cortical hormones under the stimulation of adrenocorticotropic hormone.

The production of glycosuria in normal rats with adrenocorticotropic hormone injections has recently been achieved by Ingle *et al.* (1946).

Male rats were force-fed a high carbohydrate diet and developed hyperglycemia and glycosuria through adrenal stimulation by the adrenocorticotropic hormone. There was an accompanying loss of weight and an increased excretion of urinary nitrogen and of potassium. Similar results were obtained with 17-hydroxycorticosterone (Ingle, Sheppard *et al.*, 1945). The experiments indicate that the adrenal cortex, after stimulation with adrenocorticotropic hormone, increases its production and secretion of the steroids which affect carbohydrate metabolism. It is not yet clear as to whether electrolyte metabolism is also under the control of the adrenocorticotropic hormone.

The diabetogenic action of adrenocorticotropic hormone has furthermore been demonstrated in alloxan-induced diabetic rats (Bennett and Li, 1946). The results clearly show that the hormone enhances the diabetes and opposes insulin in its effects on nitrogen and glucose excretion.

It has also been shown that adrenocorticotropic hormone exercises a beneficial effect on the resistance of normal rats to cold (unpublished work) and to low atmospheric pressure (Li and Herring, 1945) and that the hormone greatly enhances the work performance of hypophysectomized rats (Ingle, Li *et al.*, 1944).

d. Effect on Lymphoid Tissues and Serum Proteins.[5] Adrenalectomy has been shown to cause involution of the thymus gland and the lymph nodes (Banting and Gairns, 1926; McMahon and Zwemer, 1929; Reinhardt and Holmes, 1940; Schacher, Browne *et al.*, 1937). It has further been demonstrated that the presence of the adrenal cortex is necessary for the involution of these tissues (Selye, 1946). In man, Addison's disease is frequently accompanied by thymic and lymph node enlargements (Guttman, 1930) and by a lymphocytosis (de las Balze, Reifenstein *et al.*, 1946). The same tendency to lymphocytosis has been noted in adrenalectomized animals (Corey and Britton, 1932; Zwemer and Lyons, 1928; White and Dougherty, 1945a,b).

The administration of adrenocorticotropic hormone to animals, on the other hand, produces a rapid involution of the thymus and lymph nodes with histological evidence of depletion and dissolution of lymphocytes (Dougherty and White, 1943, 1944, 1945; White and Dougherty, 1945a,b; Simpson, Li *et al.*, 1943; Reinhardt, Aron *et al.*, 1944). Dougherty and White (1944) have correlated this histological picture with changes in the blood lymphocyte level in the mouse, rat, rabbit, dog and human. The production of a lymphopenia in these forms is relatively rapid, the fall in lymphocytes reaching its lowest level within 6–9 hours and returning to normal within 24 hours after a single injection. These results were confirmed in the rat and dog (Reinhardt, Aron *et al.*, 1944). Further investigations on rat thoracic duct lymph showed that stimulation of the adrenal cortex by adrenocorticotropic hormone resulted in a decrease in the number of lymphocytes being delivered to the blood stream in this way, accounting at least in part for the observed blood lymphopenia (Reinhardt and Li, 1945). Thymic and lymph node involution and blood and thoracic duct lymphopenia were not produced by injections of the hormone in the absence of the adrenal, whereas it has been shown that adrenal cortical extracts and corticosteroids were effective in the absence of the adrenals (Dougherty and White, 1944). Other purified pituitary hormones did not produce these characteristic effects (Dougherty and White, 1943; Reinhardt, Aron *et al.*, 1944; Reinhardt and Li, 1945). Certain cortical steroids, furthermore, have been shown to produce lymphocyte degeneration *in vitro* (Heilman, 1945).

---

[5] We are indebted to Dr. W. O. Reinhardt for his helpful suggestions on this section.

The marked dissolution of lymphocytes and the corresponding blood lymphopenia produced by the adrenocorticotropic hormone or by administration of adrenal cortical substances was correlated by Dougherty, White, and Chase (1944) with an increase in certain antibody titers in the immunized animals. Further observations indicated that the antibodies were present in lymphocytes and that their liberation from these cells was under adrenal cortical control (Dougherty, Chase, and White, 1944, 1945). Evidence has been advanced by Dougherty and White (1944, 1945) that the rise in serum proteins following adrenocorticotropic hormone administration, or by the injection of adrenal cortical hormones, is the result of elevation of the antibody-containing serum globulins, but this has not received confirmation. The prior work of Levin and Leathem (1942), confirmed in this laboratory (Li and Reinhardt, 1947), demonstrated that adrenal cortical extracts or adrenal cortical stimulation by adrenocorticotropic hormone *in the rat* is not attended by a rise in serum globulins. There is, in fact, a definite rise in serum albumin. Dougherty and White (1945) employed rabbits in their experimental investigations. It is, however, doubtful that the difference in effects can be related to species differences, since the behavior of the lymphocytes in response to adrenal cortical secretions is identical in both animal forms. The finding that the serum-globulin fraction in the rat was not elevated does not necessarily negate the conclusion that the antibody-containing portion of the globulin may be increased relative to the nonantibody-containing globulins, since it is impossible to differentiate antibody-containing and nonantibody-containing globulin by present physicochemical methods.

e. Effect on Cholesterol and Ascorbic Acid Content of the Adrenal. Sayers, Sayers *et al.* (1944, 1946) reported that a single dose of adrenocorticotropic hormone causes a decrease of cholesterol in the adrenals of normal rats and guinea pigs within a period of a few hours and that the cholesterol level tends to return to normal 24 hours after hormone treatment. In hypophysectomized rats, similar effects on adrenal cholesterol occurs if the hormone injection is begun 3 days after the operation; the decrease in adrenal cholesterol content can not be observed after a longer postoperative interval. It is significant that the fall of cholesterol is accompanied by a rise in liver glycogen.

Similar studies on the ascorbic acid content of the adrenal have been made by the Yale group (Sayers, Sayers *et al.*, 1946). It was found that the injection of adrenocorticotropic hormone into rats and guinea pigs produces a prompt fall in adrenal ascorbic acid. The return of adrenal ascorbic acid in the rat to a normal level is quite rapid but the level in the guinea pig remains subnormal even 24 hours after the injection.

From these results the authors suggested that cholesterol is a possible precursor of the adrenal cortical steroids and that the release of these steroid hormones has a special relation to the decrease in the adrenal ascorbic content. Recently Lowenstein and Zwemer (1946) isolated a new steroid from aqueous extracts of the adrenal glands. The new compound contains a molecule of ascorbic acid and affects carbohydrate metabolism in a manner resembling dehydrocorticosterone. The lowering of ascorbic acid in the adrenal caused by adrenocorticotropic hormone stimulation as found by Sayers *et al.* may possibly be due to increment in the ascorbic acid-steroid complex secretion. If this is the case, it should be possible to isolate a similar ascorbic acid-steroid complex from the circulating blood.

REFERENCES

1. Astwood, E. B., and Tyslowitz, R. 1942. *Federation Proc.* **1,** 4.
2. De la Balze, F. A., Reifenstein, E. C., Jr., and Albright, F. 1946. *J. Clin. Endocrinol.* **6,** 312.
3. Banting, F. G., and Gairns, S. 1926. *Am. J. Physiol.* **77,** 100.
4. Bates, R. W., Riddle, O., and Miller, R. A. 1940. *Endocrinology* **27,** 781.
5. Becks, H., Simpson, M. E., and Evans, H. M. 1945. *Anat. Record* **92,** 121.
6. Becks, H., Simpson, M. E., Evans, H. M., Ray, R. D., Li, C. H., and Asling, C. W. 1946. *Anat. Record* **94,** 631.
7. Becks, H., Simpson, M. E., Li, C. H., and Evans, H. M. 1944a. *Endocrinology* **34,** 305.
8. Becks, H., Simpson, M. E., Marx, W., Li, C. H., and Evans, H. M. 1944b. *Endocrinology* **34,** 311.
9. Bennett, L. L., Applegarth, A. P., and Li, C. H. 1946. *Proc. Soc. Exptl. Biol. Med.* **62,** 42.
10. Bennett, L. L., and Li, C. H. 1946. *Endocrinology* **39,** 63.
11. Bierring, E., and Nielsen, E. 1932. *Biochem. J.* **26,** 1015.
12. Bulbring, E. 1938. *Quart. J. Pharm. Pharmacol.* **11,** 26.
13. Burtner, E. 1943. *J. Am. Chem. Soc.* **65,** 1238.
14. Ciereszko, L. S. 1945. *J. Biol. Chem.* **160,** 585.

15. Collip, J. B., Anderson, E. M. and Thomson, D. L. 1933. *Lancet* **2,** 347.
16. Collip, J. B., Selye, H., and Thomson, D. L. 1933. *Proc. Soc. Exptl. Biol. Med.* **30,** 544.
17. Corey, E. L., and Britton, S. W. 1932. *Am. J. Physiol.* **102,** 699.
18. Davidson, C. S., and Moon, H. D. 1936. *Proc. Soc. Exptl. Biol. Med.* **35,** 281.
19. Dingemanse, E., and Freud, J. 1935. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **5,** 109.
20. Dott, N. M., and Fraser, J. 1923. *Quart. J. Exptl. Physiol. (Suppl.)* **13,** 107.
21. Dougherty, T. F., Chase, J. H., and White, A. 1944. *Proc. Soc. Exptl. Biol. Med.* **57,** 295.
22. Dougherty, T. F., Chase, J. H., and White, A. 1945. *Proc. Soc. Exptl. Biol. Med.* **58,** 135.
23. Dougherty, T. F., and White, A. 1943. *Proc. Soc. Exptl. Biol. Med.* **53,** 132.
24. Dougherty, T. F., and White, A. 1944. *Endocrinology* **35,** 1.
25. Dougherty, T. F., and White, A. 1945. *Am. J. Anat.* **77,** 81.
26. Dougherty, T. F., White, A., and Chase, J. H. 1944. *Proc. Soc. Exptl. Biol. Med.* **56,** 28.
27. Downs, W. G., Jr. 1930. *J. Dent. Research* **10,** 601.
28. Evans, H. M., Cornish, R. E., and Simpson, M. E. 1929. *Proc. Soc. Exptl. Biol. Med.* **27,** 101.
29. Evans, H. M., and Long, J. A. 1921a. *Anat. Record* **21,** 56.
30. Evans, H. M., and Long, J. A. 1921b. *Anat. Record* **21,** 62.
31. Evans, H. M., Meyer, K., and Simpson, M. E. 1933. *Mem. Univ. Calif.* **11,** 59.
32. Evans, H. M., and Simpson M. E. 1925. *Anat. Record* **29,** 356.
33. Evans, H. M., and Simpson, M. E. 1931. *Am. J. Physiol.* **98,** 511.
34. Evans, H. M., Simpson, M. E., and Li, C. H. 1943. *Endocrinology* **33,** 237.
35. Evans, H. M., Simpson, M. E., and Li, C. H. 1946. *Endocrinology* **39,** 71.
36. Evans, H. M., Simpson, M. E., Marx, W., and Kibrick, E. 1943. *Endocrinology* **32,** 13.
37. Evans, H. M., Uyei, N., Bartz, Q. R., and Simpson, M. E. 1939. *Endocrinology* **22,** 483.
38. Fevold, H. L., Lee, M., Hisaw, F. L., and Cohn, E. J. 1940. *Endocrinology* **26,** 999.
39. Fraenkel-Conrat, H., Meamber, D. L., Simpson, M. E., and Evans, H. M. 1940. *Endocrinology* **27,** 605.
40. Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M. 1940a. *Ann. facultad med. Montevideo* **25,** 617.
41. Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M. 1940b. *Endocrinology* **27,** 809.
42. Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M. 1940c. *Endocrinology* **23,** 793.
43. Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M. 1943. *Am. J. Physiol.* **138,** 439.
44. Fraenkel-Conrat, J., Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M. 1942. *Am. J. Physiol.* **137,** 200.
45. Freud, J., Levie, L. H., and Kroom, D. B. 1939. *J. Endocrinol.* **1,** 56.
46. Gaebler, O. H. 1933. *J. Exptl. Med.* **57,** 349.
47. Gaebler, O. H. 1946. Private communication.
48. Gordan, G. S., Li, C. H., and Bennett, L. L. 1946a. Unpublished.
49. Gordan, G. S., Li, C. H., and Bennett, L. L. 1946b. *Proc. Soc. Exptl. Biol. Med.* **62,** 103.

50. Creep, R. O., van Dyke, H. B., and Chow, B. F. 1940. *J. Biol. Chem.* **133**, 289.
51. Guttman, G. P. 1930. *Arch. Path.* **10**, 742, 895.
52. Handelman, M. B., and Gordon, E. F. 1930. *J. Pharmacol.* **38**, 349.
53. Harrison, H. C., and Long, C. N. H. 1940. *Endocrinology* **26**, 971.
54. Heilman, H. 1945. *Proc. Staff Meetings Mayo Clinic* **20**, 310.
55. Herring, V. V., and Evans, H. M. 1943. *Am. J. Physiol.* **140**, 452.
56. Holder, D. 1937. *Arch. anat. histol. embryol.* **24**, 1.
57. Houssay, B. A., and Leloir, L. F. 1935. *Rev. soc. arg. biol.* **11**, 464.
58. Ingle, D. J., Higgins, S. M., and Kendall, E. C. 1938. *Anat. Record* **71**, 363.
59. Ingle, D. J., Li, C. H., and Evans, H. M. 1944. *Endocrinology* **35**, 91.
60. Ingle, D. J., Li, C. H., and Evans, H. M. 1946. *Endocrinology* **39**, 32.
61. Ingle, D. J., Sheppard, R., Evans, J. S., and Kuizenga, M. H. 1945. *Endocrinology* **37**, 341.
62. Kibrick, E. A., Becks, H., Marx, W., and Evans, H. M. 1941. *Growth* **4**, 437.
63. Kleiber, M., and Cole, H. H. 1939. *Am. J. Physiol.* **125**, 747.
64. Lee, M. O., and Schaffer, N. K. 1934. *J. Nutrition* **7**, 337.
65. Leonard, S. L. 1944. *Endocrinology* **35**, 83.
66. Levin, L., and Leathem, J. H. 1942. *Am. J. Physiol.* **136**, 306.
67. Lewis, J. C., and Olcott, H. S. 1945. *J. Biol. Chem.* **157**, 265.
68. Li, C. H. 1946a. *Federation Proc.* **5**, Part 2, 144.
69. Li, C. H. 1946b. Unpublished data.
70. Li, C. H. 1947. *J. Phys. Colloid Chem.* **51**, 218.
71. Li, C. H., and Evans, H. M. 1944. *Science* **99**, 183.
72. Li, C. H., Evans, H. M., and Simpson, M. E. 1945. *J. Biol. Chem.* **159**, 353.
73. Li, C. H., and Herring, V. V. 1945. *Am. J. Physiol.* **143**, 548.
74. Li, C. H., Kalman, C., Evans, H. M., and Simpson, M. E. 1946. *J. Biol. Chem.* **163**, 715.
75. Li, C. H., Lyons, W. R., and Evans, H. M. 1940a. *J. Gen. Physiol.* **23**, 433.
76. Li, C. H., Lyons, W. R., and Evans, H. M. 1940b. *J. Biol. Chem.* **136**, 709.
77. Li, C. H., Lyons, W. R., and Evans, H. M. 1941a. *J. Gen. Physiol.* **24**, 303.
78. Li, C. H., Lyons, W. R., and Evans, H. M. 1941b. *J. Biol. Chem.* **140**, 43.
79. Li, C. H., and Reinhardt, W. O. 1947. *J. Biol. Chem.* **167**, 487.
80. Li, C. H., Simpson, M. E., and Evans, H. M. 1940a. *Science* **92**, 355.
81. Li, C. H., Simpson, M. E., and Evans, H. M. 1940b. *Endocrinology* **27**, 803.
82. Li, C. H., Simpson, M. E., and Evans, H. M. 1942a. *J. Am. Chem. Soc.* **64**, 368.
83. Li, C. H., Simpson, M. E., and Evans, H. M. 1942b. *Science* **96**, 450.
84. Li, C. H., Simpson, M. E., and Evans, H. M. 1943. *J. Biol. Chem.* **149**, 413.
85. Li, C. H., Simpson, M. E., and Evans, H. M. 1946. *Arch. Biochem.* **9**, 259.
86. Long, C. N. H. 1943. *Ann. N. Y. Acad. Sci.* **XLIII**, 383.
87. Long, C. N. H., and Katzin, B. 1938. *Proc. Soc. Exptl. Biol. Med.* **38**, 566.
88. Lowenstein, B. E., and Zwemer, R. L. 1946. *Endocrinology* **39**, 63.
89. Lyons, W. R. 1937. *Proc. Soc. Exptl. Biol. Med.* **35**, 645.
90. McMahon, H. E., and Zwemer, R. L. 1929. *Am. J. Path.* **5**, 491.
91. Marx, W., Magy, D. B., Simpson, M. E., and Evans, H. M. 1942. *Am. J. Physiol.* **137**, 544.
92. Marx, W., Simpson, M. E., Evans, H. M. 1942a. *Endocrinology* **30**, 1.
93. Marx, W., Simpson, M. E., and Evans, H. M. 1942b. *J. Biol. Chem.* **147**, 77.
94. Marx, W., Simpson, M. E., and Evans, H. M. 1944. *Proc. Soc. Exptl. Biol. Med.* **55**, 250.

95. Marx, W., Simpson, M. E., Li, C. H., and Evans, H. M. 1943. *Endocrinology* **33,** 102.
96. Mirsky, I. A. · 1939. *Endocrinology* **25,** 52.
97. Moon, H. D. 1937a. *Proc. Soc. Exptl. Biol. Med.* **35,** 649.
98. Moon, H. D. 1937b. *Proc. Soc. Exptl. Biol. Med.* **37,** 34.
99. Moon, H. D. 1940. *Proc. Soc. Exptl. Biol. Med.* **43,** 42.
100. Noble, R. L., and Collip, J. B. 1941. *Endocrinology* **29,** 934.
101. Parkes, A. S. 1945. *Physiol. Revs.* **25,** 203.
102. Ray, R. D., Evans, H. M., and Becks, H. 1941. *Am. J. Path.* **17,** 509.
103. Reichstein, T. 1936. *Helv. Chim. Acta* **19,** 223.
104. Reichstein, T., and von Euw, J. 1938. *Helv. Chim. Acta* **21,** 1197.
105. Reinhardt, W. O., Aron, H., and Li, C. H. 1944. *Proc. Soc. Exptl. Biol. Med.* **57,** 19.
106. Reinhardt, W. O., and Holmes, R. O. 1940. *Proc. Soc. Exptl. Biol. Med.* **45,** 267.
107. Reinhardt, W. O., and Li, C. H. 1945. *Science* **101,** 360.
108. Reiss, M., Balin, J., Oestreicher, F., and Aronson, V. 1936. *Endokrinologie* **18,** 1.
109. Russell, J. A. 1938. *Am. J. Physiol.* **121,** 755.
110. Russell, J. A. 1942. *Am. J. Physiol.* **136,** 95.
111. Sayers, G., Sayers, M. A., Fry, E. G., White, A., and Long, C. N. H. 1944. *Yale J. Biol. Med.* **16,** 361.
112. Sayers, G., Sayers, M. A., Liang, T. Y., and Long, C. N. H. 1946. *Endocrinology* **38,** 1.
113. Sayers, G., White, A., and Long, C. N. H. 1943. *J. Biol. Chem.* **149,** 425.
114. Schacher, J., Browne, J. S. L. and Selye, H. 1937. *Proc. Soc. Exptl. Biol. Med.* **36,** 488.
115. Scow, R. O., and Marx, W. 1945. *Anat. Record* **91,** 227.
116. Selye, H. 1946. *J. Clin. Endocrinol.* **6,** 117.
117. Shedlovsky, T., Rothen, A., Greep, R. O., van Dyke, H. B., and Chow, B. F. 1940. *Science* **92,** 178.
118. Shipley, R. A. 1942. *Endocrinology* **31,** 629.
119. Shipley, R. A., and Long, C. N. H. 1938. *Biochem. J.* **32,** 2242.
120. Simpson, M. E., Evans, H. M., and Li, C. H. 1943. *Endocrinology* **33,** 261.
121. Simpson, M. E., Li, C. H., Reinhardt, W. O., and Evans, H. M. 1943. *Proc. Soc. Exptl. Biol. Med.* **54,** 135.
122. Simpson, M. E., Marx, W., Becks, H., and Evans, H. M. 1944. *Endocrinology* **35,** 234.
123. Smith, P. E. 1930. *Am. J. Anat.* **45,** 205.
124. Teel, H. M. 1929. *Science* **69,** 405.
125. Teel, H. M., and Watkins, O. 1929. *Am. J. Physiol.* **89,** 662.
126. van Dyke, H. B., and Wallen-Lawrence, Z. 1930. *J. Pharmacol.* **40,** 413.
127. van Wagenen, G. 1928. *Am. J. Physiol.* **84,** 468.
128. Wadehn, F. 1932. *Biochem. Z.* **255,** 188.
129. Weil, L. 1941. *J. Biol. Chem.* 138, 375.
130. Wells, H. B., and Kendall, E. C. 1940. *Proc. Staff Meetings Mayo Clinic* **15,** 324.
131. White, A. 1945. The Chemistry and Physiology of Hormones, Science Press, Lancaster, p. 1.
132. White, A., Bonsnes, R. W., and Long, C. N. H. 1942. *J. Biol. Chem.* **143,** 447.

133. White, A., Catchpole, H. R., and Long, C. N. H. 1937. *Science* **86**, 82.
134. White, A., and Dougherty, T. F. 1944. *Proc. Soc. Exptl. Biol. Med.* **56**, 26.
135. White, A., and Dougherty, T. F. 1945a. *Endocrinology* **36**, 16.
136. White, A., and Dougherty, T. F. 1945b. *Endocrinology* **36**, 207.
137. Wilkens, W. E., and Regen, E. N. 1935. *Proc. Soc. Exptl. Biol. Med.* **32**, 1373.
138. Young, F. G. 1939. *J. Endocrinol.* **1**, 339.
139. Young, F. G. 1945. *Biochem. J.* **39**, 515.
140. Zwemer, R. L., and Lyons, C. 1928. *Am. J. Physiol.* **86**, 545.

Effect of Exogenous Estrogens on the Male Mammal[1]

By C. W. EMMENS and A. S. PARKES

CONTENTS

		Page
I.	Introduction	233
II.	Chemical Structure of Estrogens	234
III.	Endogenous Estrogens in the Male	234
IV.	The General Effects of Estrogens on Adult Males	236
	1. The Skeleton	236
	2. External Genitalia and the Skin	238
	3. Internal Genitalia	239
	4. The Ejaculate	240
V.	The General Effects of Estrogens on Immature Males	241
	1. Sex Differentiation	241
	2. Postnatal Development	242
VI.	Effects on the Pituitary Gland	248
	1. Morphological and Histological Changes	248
	2. Changes in the Content of Gonadotropic[2] Hormones	250
VII.	Direct Effects on the Accessory Reproductive Organs	251
	1. Seminal Vesicles and Prostate	251
	2. Mammary Gland	259
VIII.	Effects on the Adrenal Glands	262
IX.	Interaction of Estrogenic and Androgenic Substances	263
	1. Protection of Males from the Effects of Estrogens	263
	2. Synergistic Actions of Estrogens and Androgens	266
	References	267

I. INTRODUCTION

This review deals with the effects of estrogens on male mammals. Effects on birds have been dealt with recently by Parkes and Emmens (1944). Clinical aspects of the administration of estrogens are discussed by Shorr elsewhere in this volume. Except for introductory remarks, the present paper is further restricted to a discussion of the action of chemically pure substances; the earlier work involving the use of crude extracts of gonads or urine has already been extensively reviewed by Koch (1937–38), Burrows (1945), and others.

---

[1] Some of the material in this review has been taken from a manuscript entitled "Effects of Estrogens and Androgens on the Male" prepared by us early in 1939 for Vol. III of *Ergebnisse der Vitamin- und Hormonforschung.* The outbreak of war made it impossible to return the corrected proof to Germany and, so far as we have been able to find out, the article was never published.

[2] Editors' Note: There is considerable difference of opinion, both in England and in America, in regard to the spelling of "gonadotropic," and other corresponding words. The editors have decided to adopt "tropic" throughout this volume, in accordance with the etymology of the suffix, which refers to turning or changing in response to a stimulus, while "trophic" refers to nutrition. The former is also consistent with the policy of Volumes III and IV and with Chemical Abstracts.

II. CHEMICAL STRUCTURE OF ESTROGENS

The preparation and relative activities of the chief naturally-occurring and synthetic estrogens are discussed in the papers cited above, and also by Marrian (1938). The substances with which most of the experimental work has been conducted, and with which we are mainly concerned here, are listed below.

α-Estradiol (Estratriene-3,17-diol), is elaborated in the ovaries, although it is perhaps not the only estrogen formed therein. Estradiol is converted in the body to *estrone* and *estriol,* which are the chief excreted estrogens. Some estradiol, however, may be excreted unchanged, notably in labor (Huffman *et al.*, 1940). β-Estradiol, the stereoisomer of α-estradiol, is found in the urine of pregnant mares, and is considerably less active as an estrogen.

Estrone (Estratriene-3-ol-17-one) is found mainly in urine, and is less active than α-estradiol. The ratio of the activities of the two substances varies with the test method between about 4 and 12.

Estriol (Estratriene-3,16,17-triol), also found in urine, is less active than estrone by the usual test methods. The relative activities are, however, greatly influenced by such factors as the number of injections and the site of administration. Ratios between 3:1 and 250:1 have been reported.

Diethylstilbestrol (4,4′-dihydroxy-α,β-diethylstilbene) is the best known of a series of highly potent synthetic estrogens prepared by Dodds and his co-workers. Its activity is between that of estrone and that of estradiol; the exact ratio again varies according to the test method. It has not been found in nature.

Hexestrol (4,4′-dihydroxy-γ,δ-diphenyl-*n*-hexane), also prepared by Dodds, has about the same potency as diethylstilbestrol. Both of these synthetic compounds differ from the natural estrogens in being highly active by mouth, but otherwise resemble them in biological action to a remarkable degree. Reports of qualitative difference in activity between these synthetic estrogens and members of the estradiol series have not been substantiated.

III. ENDOGENOUS ESTROGENS IN THE MALE

The present review deals almost exclusively with the effects of exogenous estrogens administered to the male. These effects are many and varied, but they do not necessarily show that estrogens have any essential role in the physiological economy of the normal male.

The normal male mammal is subjected to the action of estrogens in two different ways. Firstly, there is no doubt that the maternal estrogens traverse the placenta in many species and both male and female young at birth may show considerable signs of estrogenization. Such effects are naturally more obvious in female young, in which a state approaching premature puberty, the *crise genitale* of Courrier, may sometimes be seen. Transient abnormalities in male young, ascribable to the action of maternal hormones, if not toithat of maternal estrogens alone, include stratification of prostatic epithelium (Burrows, 1935a) and activity of the mammary tissue in newborn human males.

Secondly, endogenous estrogens seem to be present in normal males of many, if not all, species of mammal. In man, both androgens and estrogens are excreted by the two sexes, but whereas there is little difference between the amounts of androgen found in the urine of men and women, the excretion of estrogens by normal adult men is lower than that by normal women (Callow *et al.*, 1939; Dingemanse, Borchardt, and Laqueur, 1937; Gallagher *et al.*, 1937). Stallions' urine, however, has relatively enormous quantities of estrogen, varying from 10,000 to 400,000 "mouse units" per liter, which is as much as is excreted by the pregnant mare (Zondek, 1934). In spite of this flood of estrogen, it is to be doubted whether the stallion shows any effects known to be characteristic of estrogenized males.

Part of the estrogen excreted in the urine of males is no doubt a metabolic product of androgenic or cortical substances. However, the testes of the stallion, pig, horse, bull and man (Laqueur *et al.*, 1927; Courrier, 1934) contain varying amounts of estrogen. Horse testes are said to be richer in this respect than any other known tissue, and it is likely that the estrogen in the urine of the stallion is of gonadal origin. Estrogen is also produced by the adrenal gland of both sexes; estrone was isolated by Beall (1939, 1940) from ox adrenals. It is, therefore, unlikely that the estrogen produced in the male results solely from the metabolism of androgens.

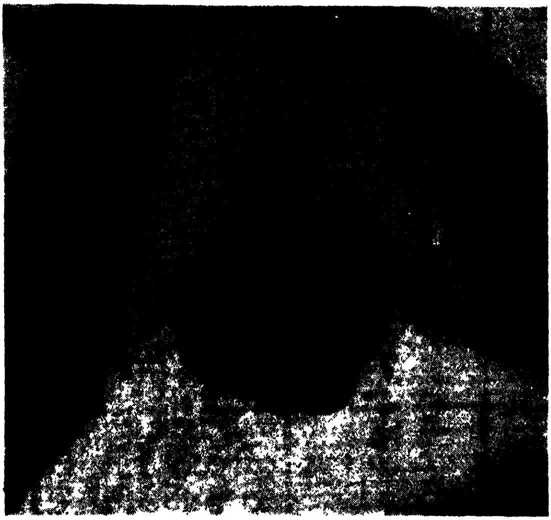
If it is postulated that the action of small amounts of estrogen on the male may be physiologically normal, the so-called cooperative and antagonistic effects of androgens and estrogens become important. A typical example of "cooperation" is the enhancement of activity of androgens on the preputial gland of gonadectomized male or female rats when an estrogen is also given in small doses (van der Woerd, 1938). In considering this type of experiment, it must be stressed that such synergistic or co-operative effects are often replaceable by increasing the dose of androgen (Korenchevsky *et al.*, 1935), and that the establishment of cooperation depends on the rather arbitrary definition that may be given to the term. There is, on the other hand, no doubt that a great many of the effects of androgens are antagonized by administration of estrogens and *vice versa.* These actions are reported more fully in Section IX.

In the absence of any conclusive evidence in either direction, one may conclude, provisionally, that the presence of a certain amount of estrogen in the normal male is incidental rather than functionally important, and that its entire absence would have little or no deleterious effect on the animal.

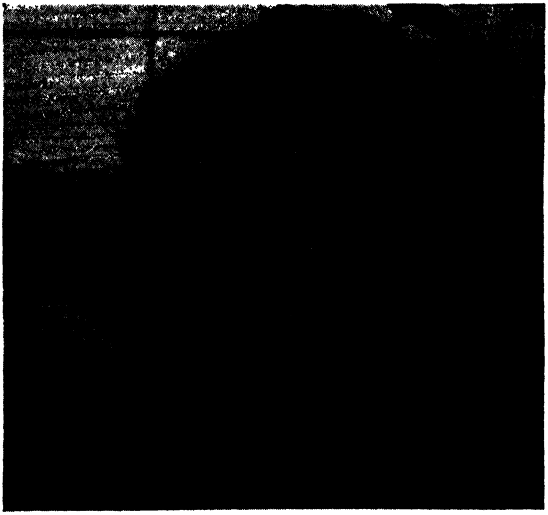
IV. The General Effects of Estrogens on Adult Males

1. *The Skeleton*

A number of remarkable gross effects have been produced in the male of lower mammals by the administration of estrogens. Burrows and Kennaway (1934) described scrotal hernias in male mice which had received estrin over a long period by inunction on the skin in benzene solution; the condition was further described by Burrows (1934a). Shortly afterwards, Gardner (1935), following up the older work with crude estrogenic extracts, observed that the administration of estrin to male mice causes decalcification of the pubic symphysis, so that the pubic bones become separated by ligaments 2–3 mm. long. The mice also showed scrotal hernia, which Gardner attributed to the changes in the pubic symphysis. Burrows (1935e) also observed separation of the pubic bones in normal and castrated male mice, but apparently regarded the hernias as an exaggeration of the normal process of testicular descent (Burrows, 1936b). Gardner (1936) found that the effects could be produced by a large number of different estrogens. A greater degree of bone resorption occurred in mice when the injections were started early in life, but the formation of scrotal hernias seemed to depend on the presence of well-developed testes, for they did not occur in mice in which testicular development had been inhibited. Similar observations were made by Burrows (1936b) who produced the hernias with every potent estrogenic compound available, if mature or nearly mature testes were present. In mice in which estrone had prevented development and descent of the testes, and in castrate mice, hernias did not appear. Further observations by Gardner and Pfeiffer (1938a) showed that endosteal bone nearly or completely replaces the marrow cavities of the femurs of mice which have received 250 I.U. or more of estrogen weekly for long periods, similar changes occurring throughout the skeleton except in the immediate vicinity of the pubic symphysis. There have been a number of subse-



(a)



(b)

Fig. 1.—Effect of prolonged administration of estriol on the external characters of the rhesus monkey. (a) Edema of the scrotum occurring in the first phase of the reaction. (b) Corunculation of the skin in the supraorbital regions as seen at the end of the second phase of the reaction. (From Bachman, Collip and Selye, 1935.)

quent studies of the effects of estrogen in increasing the weight and strength of the bones in mice or rats (*cf.* Bell and Cuthbertson, 1943).

2. External Genitalia and the Skin

The remarkable development of the sexual skin seen during the follicular phase of the cycle in monkeys can readily be produced after ovariectomy by the injection of estrogens, and experiments were soon carried out to see whether any comparable phenomenon could be produced in males. Dohrn, Hohlweg and Schoeller (1933) injected estrone into male baboons and produced a striking edema of the external genitalia. Bachman, Collip and Selye (1935) have described the effects of prolonged injection of estriol on male macaques. By 8–10 days after the beginning of injection there was great swelling and edema of the external genitalia, involving both the scrotum and the penis. Both immature and mature males develop "huge, pendent, tense scrotal enlargements." In adults the swelling of the prepuce and penile sheath may interfere with the flow of urine. Continued injection into immature animals causes no detectable enlargement or descent of the testes. The first edematous phase lasts for a period varying up to 10 days, and at its height the face is brilliantly flushed. During the next 10–40 days this initial reaction disappears more or less completely, even if treatment is continued. With further injection, a second phase sets in after a period of 20–25 days. The new reaction is a pallid or colorless edema of entirely different type and distribution; the skin is thrown up into tensely swollen ridges, intersected with irregular grooves. This reaction begins between the groin and knee, spreads over the lumbar region, and finally involves the whole dorsal and hairy part of the trunk. At the same time the skin over the supraorbital ridges becomes similarly affected. Finally the edema spreads down the legs and arms and terminates as a cuff round the ankles and wrists. This second phase may last more than 100 days, but when treatment is ultimately stopped the animal returns to normal within one month.

The edema of the region of the external genitalia of estrogenized male monkeys has also been observed by Courrier and Gros (1935a) in the Barbary ape, in which the circum-anal response is very marked, and by Parkes and Zuckerman (1935) in rhesus monkeys, in which there is great swelling of the anus, base of the tail, lower part of the back and posterior aspects of the thighs, as well as of the penis and scrotum. Zuckerman and van Wagenen (1935) found that the reaction could take place in the newborn male monkey. Extrusion of the penis has been described in estrogenized mice by Harsh, Overholser and Wells (1939).

Hair growth is retarded or even prevented by the administration of estrogen to the male (or female) mammal. A weekly dose of from 133 γ to 333 γ of estradiol benzoate given to fox terriers of either sex arrested the regrowth of hair after clipping (Gardner and De Vita, 1940). Similar results were obtained with diethylstilbestrol in dogs (Mulligan, 1943), and various natural and synthetic estrogens in rats (Emmens, 1942; Hooker and Pfeiffer, 1943).

3. *Internal Genitalia*

Prolonged injection of estrone or estriol into male rats lowers the body weight and causes testicular atrophy, followed by atrophy of the prostate and Cowper's glands and decreased fertility (Wade and Doisy, 1935). Korenchevsky and Dennison (1934b, 1935) injected daily doses of up to 18 γ of estrone for 7–43 days into normal male rats, and found depressed development of the testes without marked histological changes, and a decrease in the gain in body weight This decrease could be only partly explained by the coincident decreased appetite. del Castillo and Pinto (1937) found that adult rats receiving 15 units of estrone per day for long periods (1–4 months) may show atrophic testes with no spermatogenesis, but normal or even hypertrophied interstitial tissue. The seminal vesicles had hypertrophied muscle coats and atrophic epithelium, and the penis was reduced. With 50 units per day for 12 days there was an increase in the weight of the seminal vesicles, prostate and testes, and increased spermatogenesis. This result is reminiscent of that obtained by Hohlweg (1934) and Mazer, Israel, and Alpers (1936) who found that a short course of estrone treatment stimulated ovarian development in immature rats and rabbits. Against the view that stimulation of the male reproductive organs may be caused by small amounts of estrogen it must be remembered that even very low dosage over a long period will cause depression of activity.

Burrows and Kennaway (1934) observed atrophy of the testis and seminal vesicles in estrogenized mice at the same time as the abnormal prostatic changes mentioned above. Lacassagne (1934a), however, failed to cause testis atrophy in mice with 12,000 I.U. of estrone and concluded that this species is much more resistant than are rats and rabbits. Changes in the histology of the mouse testis following estrin administration have been described by Burrows (1935b, 1936c); the spermatogenic function was impaired or completely repressed. Gardner (1937) injected 500 I.B.U. of estradiol benzoate per week for 76–250 days, and produced variable degrees of hyperplasia of the interstitital cells and also tubular damage, with testes from one-half to slightly under normal size. The histological changes in the testes, which occur when their development is inhibited by estrogen treatment, are reviewed by Deanesly

(1939). Interstitial cell changes can be seen within 14 days of the implantation of a tablet of estrone. The nuclei shrink, the cytoplasm becomes vacuolated and the cell outlines are lost, and eosinophil colloid appears in the intertubular spaces.

The hyperplasia which occurs in the Leydig cells of the testes of some strains of mice, after prolonged injection of estrogens (Burrows, 1935d, 1936c, 1937) has been studied by Gardner (1937) and Bonser and Robson (1940), who agree in reporting that Strong A mice are particularly susceptible to this effect, other strains showing none (C_3H, CBA, N, F). In the Strong A mice the testes did not atrophy after a dosage of 3 mg. per week of triphenylethylene. In some of the mice, spermatogenesis continued, and there was, in all, an absence of the squamous metaplasia and keratinization in the accessory genital organs, typical of estrogen treatment. It is suggested that, in these mice, an increased androgen secretion occurs, which offsets in part the effects of estrogen. In each of 8 Strong A mice treated as above for 50 weeks, bilateral interstitial cell tumors were developed (Bonser, 1942) Hooker and Pfeiffer (1942) induced many testicular tumors in this strain of mice with estradiol and stilbestrol given for 8 months or longer. There is little doubt that the testicular atrophy seen after estrogenization of adult males is due to depression of pituitary gonadotropic activity (see Section VI). The atrophy of the accessory organs, notably of the prostate and seminal vesicles, is no doubt due mainly to the testicular atrophy, but also in part to the inhibition at the site of action of such androgen as may be produced by the atrophic testis (see Section IX). The remarkable effect of estrogenization in human prostatic cancer (*cf.* Dodds, 1944) is also probably due to the same combination of effects.

4. The Ejaculate

Huggins and Clark (1940) reduced the volume of the ejaculate of dogs, by the injection of 0.6 mg. or more per day of diethylstilbestrol, to almost nil. This reduction was accompanied by a rapid fall in the sperm count, which became nil after about a month. The prostate gland was atrophic in these dogs. Similar effects, apart from the decrease in sperm count, were obtained by Wallace (1946) in estrogenized boars.

A decrease in the volume of semen produced by five men injected with from 6–12,000 units of estradiol benzoate 3–6 times weekly is reported by Heckel and Steinmetz (1941a). Between 30 and 100 days clapsed before the treatment started to reduce seminal volume, which fell in all five patients to between one-half and one-quarter of its pre-injection level. In a further paper (Heckel and Steinmetz, 1941b) these authors reported that their patients lost libido during treatment, developed azoospermia and underwent a gradual enlargement of the breasts and nipples. None of these effects persisted after treatment was stopped. Mühlbock (1940), however, reports that administration of estradiol benzoate does not decrease the copulation rate in androgenized castrated rats, so that the loss of libido in normal males may be secondary to changes in the pituitary gland and thence in the testis.

Min-Chueh Chang (1942) implanted two Suffolk rams with tablets of diethylstilbestrol, from which 4–5 mg. were absorbed per ram per day. Sperm production increased 7–9 days after implantation and remained high for about 5 days. The rams remained fertile during the 80–100-day experimental period. The sperm production at the end of this period was falling below the control levels, and it seems likely that had the experiment continued longer, a significant drop would have been observed. These testicular changes, and other changes secondary to them, are related to the action of estrogens on the pituitary gland, which is considered below.

V. The General Effects of Estrogens on Immature Males

1. *Sex Differentiation*

Attempts to produce feminization of the embryonic male mammal by the antenatal administration of estrogens have not been successful until recently, largely because high doses of estrogen caused abortion or resorption of the fetuses.

Greene, Burrill, and Ivy (1938) produced feminized males by the injection of 0.375–4.0 mg. of estradiol dipropionate into pregnant rats on the 13th, 14th, and 15th day of gestation. These males exhibited, in varying degrees, hypospadias, visible nipples at birth and testes placed high up in the abdomen. Inhibited development, or absence, of the prostatic diverticula, inhibition of the development of the seminal vesicles and right vas deferens, and the presence of a vagina comparable in development with that of a newly-born female were also found in the male offspring of mothers receiving the higher doses.

In similar experiments, Raynaud (1939a,b) injected 5–7.5 mg. of estradiol dipropionate, or 2 mg. of diethylstilbestrol into pregnant mice. In male offspring, besides the inhibition of full development of the Wolffian ducts and accessory organs, a well-developed uterus was sometimes present, with oviducts opening near the testes. Greene, Burrill, and Ivy (1939) also reported the presence of portions of uteri in male offspring from pregnant rats treated with a total of 12–42 mg. of diethylstilbestrol from the 12th to the 21st day of pregnancy.

Burns (1939) injected pouch young opossums with estradiol dipropionate on the 4th, 8th, and 10th day. Each animal received an average of 20–30 γ/day. Only 5 out of 12 survived to 18 days, when they were killed. Autopsy revealed that the moribund condition of the survivors was due to urinary obstruction with complete retention, caused by hyperplasia and cornification of the urogenital sinus and urethra in both sexes. The Müllerian ducts of males were more than ordinarily well-developed; the testes were atrophic. The main actions of the estrogen were inhibitory. Later work of Burns (1942) and Burns and Moore (1941a,b), in which smaller amounts of various estrogens, equivalent to 1–6 γ/day, were given by injection or inunction to the skin, showed that prostatic development was inhibited, whereas the testis was not appreciably affected. Even these dosages caused the death of many young.

Confirmation of their earlier work and an extension of the observations to a study of adult modified males followed from Greene *et al.* (1940, 1942). After the administration to the mother of up to 35 mg. of estradiol or 100 mg. of estradiol dipropionate at varying periods during pregnancy—usually from about the 12th to 21st day of gestation—22 adult genetic males were reared by foster-parents. In nearly all of these adults, nipples were present, the scrotum was absent and the penis underdeveloped and hypospadiac, with a common orifice for the urethra and vagina at its caudal base. The testes were in the position of the female gonad and a partial vagina was present, the prostate was absent and the Wolffian duct derivatives were inhibited to various degrees. The intra-abdominal testes were not feminized, and resembled congenitally cryptorchid testes in structure. No particular correlation between dosage and effect was observed in this series.

In some modified males (not necessarily adults) asymmetrical responses were obtained. In such animals, descent of the right testis was most frequently inhibited, and modification of the right Wolffian duct more pronounced. No asymmetry of effects of estrogens on the Müllerian ducts of males was observed. This is the reverse of the situation seen in birds, where the left side of the male appears to have stronger female tendencies than the right side.

As pointed out by Moore (1944) the modifications induced in the developing mammalian embryo are not such as would indicate that steroid hormones normally play a part in the development of sex. The available evidence suggests, for instance, that the normal male mammal does not produce endogenous androgen prior to birth.

2. *Postnatal Development*

Early in the work on estrogenic extracts it was shown by Steinach and Kuhn (1926), Laqueur and de Jongh (1928), and Borchardt, Dingemanse, de Jongh and Laqueur (1929), that administration of estrogens to the male damaged the testes and caused atrophic changes in the accessory reproductive organs. These findings were initially interpreted in the light of earlier experiments on the transplantation of the gonad and were referred to as "sex antagonism," "the anti-masculine effect of estrone," and so on. Moore and Price (1930, 1932), however, reviewed the older observations and carried out further experiments. Any possibility of sex hormone antagonism as the primary effect was disposed of by the observation that testicular atrophy was also caused by testis extracts, and particularly by the observation that the harmful effect of the prolonged injection of estrogenic extracts to the male could be prevented by the simultaneous administration of gonadotropic extracts of the pituitary gland. Moore and Price concluded that the effect of the estrogen was exerted by depressing the gonadotropic function of the pituitary. The action of either estrone or testosterone may, under certain conditions, be affected by the simultaneous administration of the other, but nevertheless Moore and Price's general thesis, that the primary effect of estrogen injection lies not in inhibiting the endogenous androgens, but in depressing pituitary gland activity, has been fully substantiated.

Other functions of the pituitary gland than the gonadotropic ones may be affected by prolonged injection of estrogens, as had early been suggested by the results of Bugbee and Simond (1926), and afterwards by those of Spencer, d'Amour, and Gustavson (1931) who first showed adequately that prolonged injection of estrogenic extracts depressed the growth rate as well as affecting the reproductive organs. These authors were also able (1932) to confirm the findings of Moore and Price, that the antigonadotropic effect of estrone could be overcome by the administration of gonadotropic extracts. A great deal of fruitful work has followed these earlier observarions, although a few negative results have been reported, such as those of Shumacker and Lamont (1935) and of Engel (1934), who could not demonstrate any effects of chronic administration of small amounts of estrogenic preparations on growth or on the thyrotropic, gonadotropic or adrenotropic functions of the pituitary.

Lacassagne (1934b,c) was able to arrest development of the external genital organs of male rabbits by injecting estrone from birth onwards, but no influence was demonstrable on the efferent ducts and epididymis. At six months the surviving rabbits were cryptorchid, having received 1000 to 10,000 units weekly, the dosage increasing as their age advanced. There was atrophy of the testes and arrest of spermatogenesis, but no permanent damage was done, since the tubules became normal again after injections were stopped. Other experiments on rabbits were carried out by Frazier and Mu (1935) who recorded atrophy of the testes and of the penis. A feminine dewlap appeared and the treated males were indifferent towards females. Experiments reported by Weichert and Kerrigan (1942) are of interest, not only in showing the effects of estrogens on very young animals, but also in demonstrating that estrogen administered to a lactating animal may pass into the milk. These authors injected estrogens into rats starting on the day of parturition. Lactation was not apparently affected, and the failure of the young to grow normally was probably due partly to neglect as well as to direct effects on the young. The estrogenic effects on the reproductive system of the male young were very definite and included inhibition of development of the testes and accessory organs, abnormal development of the rete tubules and cornification of the ejaculatory ducts and prostatic utricle. Schoeller and Gehrke (1933) demonstrated similar effects in rats and suggested the action of estrin on the nervous system and the pituitary as a likely cause.

Detailed observations were made by Zondek (1936a, 1937) on the depression of the growth rate and of the reproductive organs, in rats, by prolonged administration of estrone. He concluded that the amount of hormone used and the period of injection are of vital importance in determining whether a stimulatory or depressing effect on the pituitary is obtained. Zondek (1936a, 1937) records the production of eunuchoid or dwarf eunuchoid rats, depending on the length of treatment with estradiol benzoate. Rats treated at from 4–6 weeks of age with 5000 m.u. subcutaneously twice weekly, weighed 58% less than normal controls when 18–20 weeks old. Percutaneous administration was equally effective (Zondek, 1935). The dwarf animals showed bone changes with the epiphyses open until old age (Erdheim, 1935), atrophic testes and accessory organs, loss of hair, and enlarged pituitaries.

There is some slight evidence that other functions of the pituitary, in addition to the gonadotropic and growth-promoting ones, can be affected in this way. Thus, Bialet-Laprida (1933b) and Mazer, Israel, and Alpers (1936) found regression of the thyroid after prolonged estrone administration to the rat, presumably as a secondary change to disturbance of pituitary function, while Barnes, Regan, and Nelson (1933) found evidence that the diabetogenic activity of the pituitary could be depressed by estrogen treatment.

Administration of hormone by the subcutaneous implantation of a compressed tablet of the pure crystalline substance (see Section II) has recently been found to be a very convenient method for obtaining the prolonged action necessary for depression of the pituitary gland by estrogens. Thus, Deanesly and Parkes (1938) found that a single administration of estrone in the form of tablets averaging 11 mg. caused depression of the growth rate of young male guinea-pigs for 5 months. At this stage the animals were killed and were found to have rudimentary testes (Fig. 2) and accessory organs. The absorption of estrone from the tablets, which were recovered at autopsy, amounted to less than 4 mg. per animal. Detailed results on rats and mice with the same technique (Fig. 3) have been recorded by Deanesly (1939) and Deanesly and Parkes (1941).

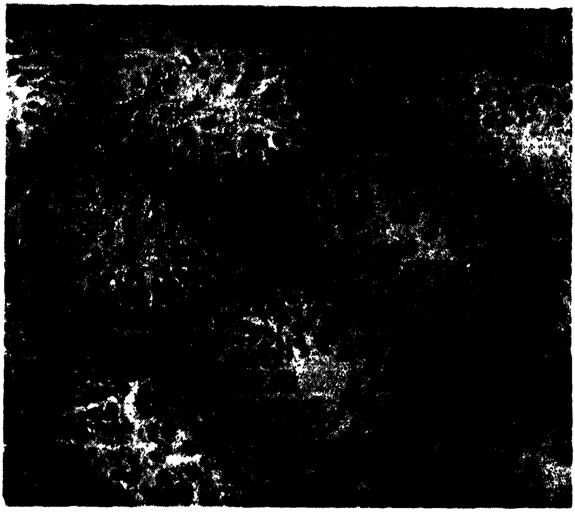


FIG. 2.—Effect of estrone on the testis of the rat. Testis of adult male rat receiving 1.6 mg. of estrone over 110 days by tablet implantation, showing degeneration of the tubules. (From Deanesly, 1939.)

This depression of pituitary activity is apparently exerted by all the naturally-occurring estrogens and also by the synthetic estrogens. Noble (1938) examined the effects on the growth rate and reproductive and endocrine organs in young male rats of the subcutaneous implantation of crystalline powder of several of the synthetic estrogens, including diethylstilbestrol, and found that the most potent retardation of growth occurred with the use of 4,4′-dihydroxy-γ,δ-diphenyl-β,δ-hexadiene (dienestrol). Noble's results are of interest in indicating a partial dissociation of estrogenic activity, as tested on ovariectomized rats, from pituitary-depressing activity as shown by the growth rate and testis size. Thus, triphenylethylene is only 1/25,000th as strong an estrogen as diethylstilbestrol in the test on ovariectomized rats, but is more than ½0th as effective as a pituitary depressant. Similarly, diethylstilbestrol is as potent an estrogen as dienestrol, but much less potent, on Noble's figures, as a pituitary depressant. It must be remembered, however, that the method of administration of the substances used by Noble did not allow for measurement of the total amount absorbed or for the possibility that absorption might have been completed and recovery begun some time before the end of the experiment.

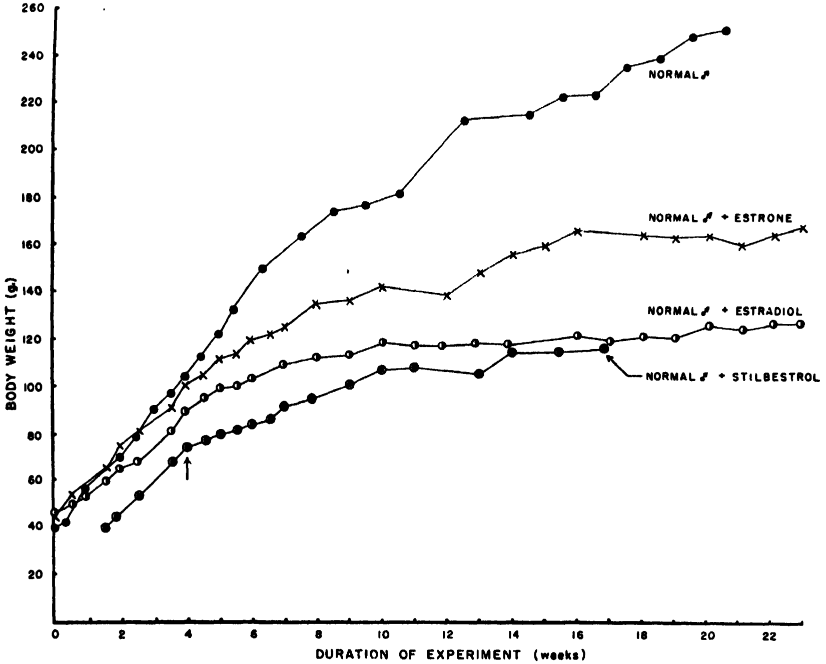


FIG. 3.—Effect of administration of estrogen on the growth-rate of young rats. Except for the rats receiving stilbestrol, in which the time of implantation is marked with an arrow, tablets were implanted at the beginning of the period of observation. (From Deanesly and Parkes, 1941.)

It should be emphasized that depression of the growth rate by the chronic administration of estrogens does not exactly parallel the effects of hypophysectomy. Young rats hypophysectomized at 40–50 g. body weight will maintain growth to 70–80 g. and then stop almost completely (Collip, Selye, and Thomson, 1933; Noble, 1938), while rats with a body weight of 70 g. or more cease to grow immediately after the operation. Rats heavily estrogenized at 40–50 g., however, continue to grow slowly and regularly to about 120 g. Gaarenstroom and Levie (1939) reported that estrone and diethylstilbestrol, as well as inhibiting pituitary activity, act directly on the epiphyseal junction. A daily dose of 280 γ of estrone, or of 10 γ of diethylstilbestrol, inhibited bone growth, and 100 γ of diethylstilbestrol completely arrested it. The authors further concluded that, whereas the effects of 250 γ of estrone daily could be nullified by the simultaneous administration of 10–15 units of growth hormone, none of the effects of diethylstilbestrol could be so abolished.

Griffiths and Young (1942) studied the effects of growth hormone-containing extracts on hypophysectomized rats and rats implanted with 15 mg. tablets of diethylstilbestrol. They found that young male rats carrying tablets of the synthetic estrogen grew at a completely normal rate if sufficient amounts of an extract of ox anterior pituitary were injected. However, the tails of these animals continued to elongate at only 40% of the normal rate, indicating that bone growth was still partially inhibited. The rate of absorption from the tablets was about 250 γ per day. Other results indicated that such a dissociation of rate of increase in body weight and the rate of elongation of the tail is not peculiar to the action of diethylstilbestrol, but was also exhibited by estrone.

Thus, the action of estrogens in inhibiting body growth would appear to be two-fold: indirect, through the pituitary, and direct, on the skeleton. The earlier establishment of centers of ossification in the female and the earlier union of epiphysis and metaphysis seen in females is in line with this latter finding. Estrin-treated male rats (Spencer, d'Amour, and Gustavson, 1932) have shorter femora than normal males, equal in length to normal females. The mechanism of this shortening has been shown both in dogs (Tausk and de Fremery, 1935) and mice (Gardner and Pfeiffer, 1938a) to be a premature union of the epiphyses. The latter authors also found a supercalcification of the femora, reported by others (Zondek, 1937; Miller, Orr, and Pybus, 1943).

Comparatively little attention has been paid to the recovery of the normal growth-rate and the male reproductive organs after the cessation of estrogen treatment. Spencer, d'Amour, and Gustavson (1932), however, were able to show that the growth-rate reverted to normal and the reproductive organs tended to return towards functional normality in rats, though only one male out of three had live spermatozoa 12 weeks after the cessation of injection. De Jongh and Laqueur (1934) report the recovery of fertility in male rats whose development has been delayed by estrin. This problem of recovery has been considered by Deanesly (1939), who found that after the removal of the tablet of estrogenic material the testes enlarged and tended to revert to normal, and might contain fully formed spermatozoa within 6 weeks in mice and after about 7 weeks in rats. Matthews, Schwabe, and Emery (1942) studied the recovery of male rats which had been fed with 1 mg. of diethylstilbestrol 3 times a week for 58 days. At the end of this period, all were sterile. Thirteen only of 17 rats recovered their fertility, in a mean time of 8 days, but histological studies showed that the testis had not fully recovered in all cases..

VI. Effects on the Pituitary Gland

1. *Morphological and Histological Changes*

It has been seen in the foregoing, that many of the effects of estrogens in the male are mediated by the pituitary gland. A great deal of work has been carried out on the morphological changes produced by estrogens in the pituitaries of normal males. As early as 1914, Berblinger (1914) reported that changes characteristic of pregnancy could be brought about in both male and female rabbits by the injection of placental extract. These results were confirmed for rabbits, rats and guinea-pigs by numerous other workers using various impure placental preparations, and the results can now be interpreted as due either to the estrogens in the extract, or to gonadotropin which might stimulate the production of androgenic or estrogenic material by the gonads. Severinghaus (1934a,b) showed that the anterior pituitary glands of male rats, injected with pregnancy urine extracts, exhibit a marked basophilic degranulation, a finding shown to be characteristic of estrogen-injected males by Wolfe and Phelps (1935). The latter authors also found that injections of 200 rat units of estradiol benzoate per day for 10 days produced a variable but definite increase in pituitary weight (*cf.* similar findings for females by Hohlweg, 1934; and Halpern and d'Amour, 1934). In the heavier glands many eosinophiles showed degranulation, and both they and the basophiles were present in relatively smaller numbers than normal, while the chromophobes increased in number and sometimes in size. The negative image of the Golgi apparatus was often enlarged in both eosinophiles and chromophobes.

An increase in weight of the pituitaries of adult male rats treated with estrone was also reported by Lipschütz, Palacios, and Akel (1936) who found a 70% average increase in pituitary weight in 31 rats receiving about 1.5 mg. of estrone in the course of 124–145 days. Cramer and Horning (1936a) reported the production of pituitary tumors in the male mouse after prolonged application of estrone or estradiol to the skin. They described the tumor as an hemorrhagic chromophobe adenoma of the anterior pituitary gland. Cramer and Horning (1936a) were able to produce chromophobe adenomata in the pituitaries of castrated male mice, similar to those produced in normal males and females. This observation was rapidly followed by that of Zondek (1936b) who described gross pituitary enlargement in estrone-treated male rats, which had received up to 340,000 mouse units in 14–19 weeks by twice-weekly applications. Females were reported not to show pituitary enlargement when similarly treated. Cramer and Horning (1936c) examined the pituitaries of their treated mice histologically, and found the enlarged anterior lobe to consist mainly of chromophobe cells, there being also intense congestion, hemorrhages and colloid production. Females in these experiments were found to be as much affected as males. These authors believed that castration renders the animal more sensitive to the effects of estrogen (1936c). Burrows (1936a) found only one much enlarged pituitary in 567 male mice treated with estrone by cutaneous application for prolonged periods.

Severinghaus (1937) summarizes the changes in the pituitary after estrogen treatment as an early enlargement of the basophiles, including the Golgi apparatus, followed by basophilic degranulation. Later, the degranulation becomes widespread and the hypertrophy of the Golgi apparatus extreme, the eosinophiles become active and undergo the same changes as the basophiles, and, after prolonged or massive dosage, the now enlarged anterior pituitary is almost devoid of granular cells. Gross tumor formation then follows continued stimulation. These changes have been followed in the rat by Wolfe and Wright (1938) who used animals injected for between one month and one year and found adenomatous changes in the pituitaries of females as well as males in the long-period experiments. They describe three types of adenomatous reaction, including adenoma associated with vascular changes, small nodular adenomatous foci, or diffuse adenomatous hyperplasia of the whole gland (mainly described from female material).

Wolfe and Wright (1938) also record pituitary adenomata in both male and female castrated rats injected for long periods with estradiol benzoate, but the two treated castrated males described did not show advanced adenomata formation, having only small nodular foci of abnormal cells. These had received estrogen treatment 257 and 325 days.

In the golden hamster (*Cricetus auratus*), the main effect of prolonged dosage with estrogens on the pituitary gland of both sexes is enlargement of the pars intermedia. This phenomenon, originally reported by Vasquez-Lopez (1944), has been confirmed by Koneff *et al.* (1946). After the implantation of 10 mg. tablets of estradiol benzoate or diethylstilbestrol (with replacements when necessary) the pars intermedia was doubled in width. By 8½ months, the pars anterior was completely invaded by cells from the pars intermedia, which was by that time grossly enlarged. The cells of the pars intermedia were, however, mostly healthy in appearance, with evidence of high activity.

Smith (1944) found that the release of gonadotropic and adrenotropic principles of the anterior pituitary of mature male rats followed the administration of a total dose of 12.6–117 γ of a lactone (an oxidative inactivation product of estrone, first produced by Westerfeld) (Westerfeld, 1942), while significant enlargement of the pituitary gland also occurred on the higher dosages. The estrogenic activity of the amounts of lactone given was equivalent to 0.85–7.8 γ of estrone, while 10–120 γ of estrone failed to produce the same responses. Therefore, Smith argues that these effects of estrone may be due to an inactivation product identical with, or similar to, that used.

Several investigators have reported partial or complete restoration to normal condition of the pituitaries of castrated male rats following the injection of estrogen, mostly in long-duration experiments. Montpellier and Chiapponi (1930) found a histological return almost to normal in the pituitaries of castrated rats injected with estrin. Hohlweg and Döhrn (1931) found that quite small doses of estrogen would prevent castration changes in the pituitary gland of immature male rats, and Nelson (1933, 1934), injecting 15–50 rat units of estrone per day for 28–48 days, also found an almost complete restoration, with very few castration cells remaining. Crooke and Korenchevsky (1935), injecting 20–180 I.U. of estrone per day for 29–43 days (personal communication), found a return towards normal, castration cells being almost completely absent, but the basophiles were still large and present in greater numbers than in normal controls. Allanson (1937) did not obtain complete correction of the pituitaries of castrated male rats within 20 days with a total dose of 1 mg. of estrone, but such a dosage was effective in preventing castration changes in newly castrated rats. Desclin (1934) injected both male and female castrated rats with up to 50 mouse units of estrin per day, commencing immediately after castration. He did not find maintenance of the normal histological appearance, the males in particular showing many castration cells when killed on the 13th to 20th day.

2. Changes in the Content of Gonadotropic Hormones

The observations that the functional gonadotropic activity of the pituitary gland seems to be decreased by chronic administration of estrogen, and that striking morphological changes take place in the gland, prompted examination of the amount of gonadotropic substance in the pituitaries of such chronically-treated animals. Burch and Cunningham (1930) found that the pituitaries of intact or castrated male rats, injected for 6 days with a small amount of estrogenic extract of placenta, showed more gonadotropic activity when implanted into immature female mice than did the pituitaries from normal control rats of the same weight. Meyer, Leonard, Hisaw, and Martin (1930) gave small amounts of estrogenic extract of amniotic fluid to female rats for 30–40 days, and found that their pituitaries contained less gonadotropic activity when tested on immature female rats, than did the pituitaries of controls. Similar results were obtained with the pituitary glands of males injected for 31 days. Leonard, Meyer, and Hisaw (1931), and Meyer, Leonard, Hisaw, and Martin (1932) reported further experiments in which the administration of estrogenic extracts to rats reduced the gonadotropic potency of the pituitary glands. It is likely, therefore, that administration of estrogen to the castrated male reduces the amount of gonadotropic hormone in the pituitary gland to normal, as reported for females by Bialet-Laprida (1933a). These authors conclude that their results harmonize with the theory that the anti-gonadal action of estrogens (Section IV) is due to depression of the gonadotropic action of the pituitary gland. Lipschütz (1934) examined the effect of estrone on the capacity of the pituitary gland to cause luteinization in the young female rat. He found that the pituitary glands of chronically estrogenized rats showed a decreased power to cause luteinization, but an increased power to stimulate estrogen production by the ovary. Lipschütz (1935) later found that, whereas the pituitary gland of the normal male rat will regularly produce heavy luteinization in the immature female, the glands from estrogenized males may not produce any luteinization.

Observations on the effect of estrogen treatment on the excretion of gonadotropic substance all seem to have been made on females, and are, therefore, outside the scope of this review.

VII. Direct Effects on the Accessory Reproductive Organs

1. *Seminal Vesicles and Prostate*

Administration of estrone to the male also causes direct changes in the accessory reproductive organs, and much work has been directed towards the experimental production of changes similar to those seen in benign enlargement of the human prostate. A few experiments have been performed on men. Hamilton, Heslin, and Gilbert (1937) gave 13 injections of from 50 to 150 γ of estrone, and found no prostatic changes as determined by clinical signs and examination of biopsy specimens. Larger doses of estrogen, however, were found by Moore and McLellan (1938) to cause stratification and occasional metaplasia of the epithelium of the urethra and collecting ducts. Similar changes, together with effects on the uterus masculinus, have been described in estrogenized newborn male infants by Sharpey-Schafer and Zuckerman (1940).

At an earlier stage, Freud (1933) had shown that both estrone and estradiol promote the development of smooth muscle in the seminal vesicles of young castrated rats (Fig. 4). In further investigations, David, Freud, and de Jongh (1938) examined the action of a large number of compounds and showed that a rough correlation existed between estrogenic and myotrophic activity. The effects were sometimes sufficient to increase the weight of the glands appreciably, although there was no stimulation of the epithelium or of secretory activity. Thus, 1 mg. of estradiol daily for 5 days doubled the weight of the rudimentary seminal vesicles. Korenchevsky and Dennison (1934a, 1935) and Korenchevsky, Dennison, and Brovsin (1936) studied the effect of estrone and estradiol on castrated rats and of estrone on normal rats. They found depressed development of the secondary sexual organs in normal rats, but increased weight of prostate and seminal vesicles, and no constant changes in the penis and preputial glands in castrated rats. They found that in castrated rats the chief histological change in the seminal vesicles is a pathological increase in the amount of fibrous tissue, while the myotrophic effect described by Freud does not exceed normal levels. The fibrotrophic effect is less pronounced in the prostate, but is greater than the myotrophic effect. Overholser and Nelson (1935) also found that injections of estrone stimulated the smooth muscle of the seminal vesicles in both normal and castrated rats.

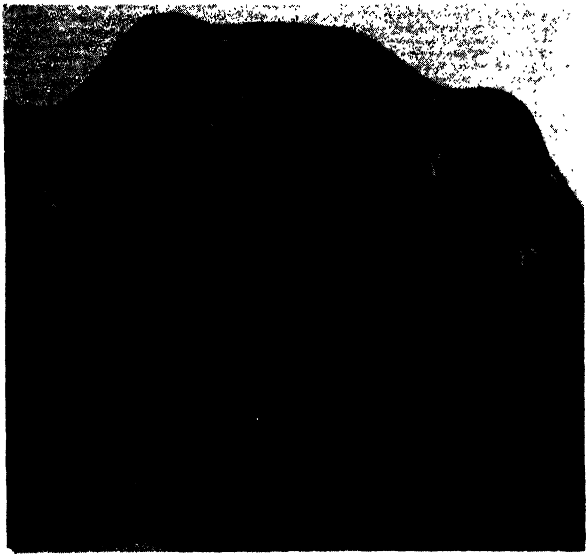


FIG. 4.—Effect of estrogen on the seminal vesicles. Seminal vesicle of castrated rat receiving 6 mg. of estrone over 25 days by tablet implantation, showing extreme hypertrophy of the fibrous tissue and degeneration of the epithelium. The seminal vesicles weighed 55 mg. as compared with 4 mg. in the untreated castrate.

This work was extended to rhesus monkeys by Parkes and Zuckerman (1935) who found that daily injections of 1 mg. or more of estrone causes extensive growth of the smooth muscle of the prostate and seminal vesicles, and a decrease in the glandular tissue. No growth of the epithelium of the vesicular lumen or of the true prostatic glands occurred. A similar result was obtained by van Wagenen (1935) in rhesus monkeys, and by Courrier and Gros (1935a) in the Barbary ape. Courrier and Gros also observed some gross enlargement of the prostate. Zuckerman and Parkes (1936a) carried out further experiments in other species of monkeys and in several of them obtained the same general increase in the fibro-muscular tissue of the seminal vesicles and prostate gland. Subsequently, Zuckerman (1938) injected two immature rhesus monkeys with estrone each day for more than a year; at the end of this period the epithelium of the true prostatic gland was still unchanged, though the prostates were greatly enlarged owing to the enormous development of fibrous tissue. Experiments on other species of mammal have given similar results. Thus, Wells (1936) found that injections of estrone into the male ground squirrel causes an increase in the amount of connective tissue and smooth muscle in the seminal vesicles, epididymis and vas deferens.

Experiments on these lines, begun by Freud in 1933, emphasize the lack of effect of estrone on the glandular epithelium of the seminal vesicles and prostate and, taken in conjunction with the well established "anti-masculine" effect of the estrogens, contrast strongly with the line of work begun by Lacassagne, also about 1933, on the induction of metaplasia of the epithelium of the prostate glands in mice. Lacassagne (1933) reported that prolonged injection of estrone into male mice often causes distention of the bladder due to compression of the urethra by accessory reproductive glands in a state of great hypertrophy. Lacassagne called attention to the discrepancy between his results and those of the early workers (Section IV) who had caused atrophy of the testes and accessory glands in rats by the administration of estrone. He carried out detailed work with normal, castrated and immature mice, administering large doses of estrone daily. After about 5 months of treatment he observed retention of urine, and consequent hydronephrosis and cachexia. Autopsy usually showed great hypertrophy of the posterior cranial lobes of the prostate, which were distended with a "magna puriform." The seminal vesicles, on the other hand, were minute appendages to this mass. The events leading up to this condition are as follows: during the first two weeks there is atrophy of the seminal vesicles and posterior prostate, while the anterior prostate is not much affected. There is then some recovery, but by the fourth month marked changes appear in the epithelium of the posterior prostatic lobes, which undergo extensive metaplasia and cornification. This metaplasia extends progressively to all the glands of the posterior prostatic lobes, which are transformed into large hollow bodies full of desquamated debris (Fig. 5). In a later paper Lacassagne and Villela (1933) consider details of the histology of the process. Burrows and Kennaway (1934) and Burrows (1934a), shortly after Lacassagne, published an account of independent experiments in which estrone was administered to mice for long periods by application to the skin in benzene solution; results identical with those of Lacassagne are recorded, together with the scrotal hernias described above and atrophy of the testes. The same reaction is produced by a large number of estrogens (Burrows, 1936b,c).

Lacassagne interpreted the response of the posterior prostate of the mouse to estrone on the grounds that derivatives of the Müllerian duct in the male might well be expected to respond to estrone by cornification, as does the vagina of the female, and that, if such derivatives occurred in male mice, they might most reasonably be expected in the posterior prostate. Burrows, however, has pointed out that the ventral lobe of the mouse prostate may also undergo keratinizing metaplasia if estrogenization is sufficiently prolonged, and that a striking feature of the response of the prostate is the gradient of sensitivity from one part to

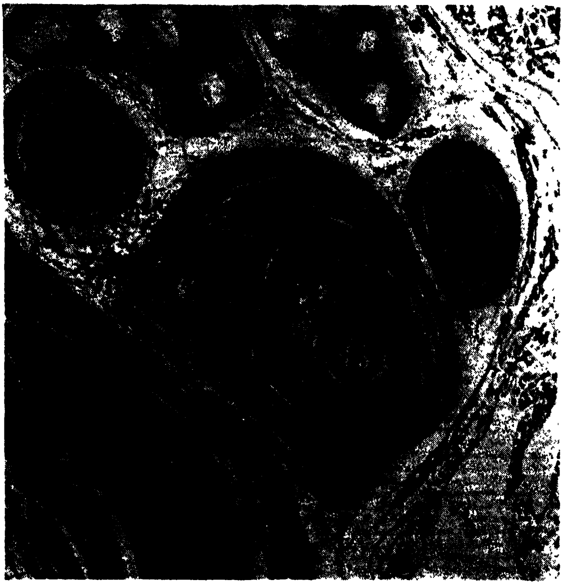


FIG. 5.—Effect of estrogen on the prostate of the mouse. Prostate of mouse receiving cutaneous application of a 0.1 % solution in benzene of equilin over 56 days, showing dilation and cornification of glands, and also the presence of a number not yet affected. (From material of Burrows.)

another. De Jongh (1935a) also came to the same conclusions after studying the effects of estrone on castrated rats and mice, and pointed out that estrogens have in the main an analogous effect in both sexes. In the light of this work certain observations made later on other species are of great interest. De Jongh and Kok (1935) described the effect of estrone on the prostate of the dog, and Zuckerman and Groome (1937) found that the keratinizing metaplasia of the glands thus produced is identical with that seen in a type of spontaneous prostatic enlargement in this species (Fig. 6). In several species of monkey (Parkes and Zuckerman, 1935; Zuckerman and Parkes, 1936a; Courrier and Gros, 1935b; van Wagenen, 1935) even short treatment with estrone will cause intense cornification of the epithelium of the uterus masculinus (Fig. 7a) and of the vera montanum, while more prolonged treatment (Zuckerman, 1938) causes metaplasia of parts of the urethra, of the terminal parts of the ejaculatory ducts and of the collecting ducts of the prostate. Fig. 7a should be compared with Fig. 7b which illustrates a uterus masculinus from an immature rhesus monkey which had received 100 γ of estrone, plus 5 mg. of androstanediol daily for 14 days; this uterus masculinus is substantially normal in appearance. In other words, the epithelial growth taking place in monkeys is confined to the utricular bed. In mice, Burrows (1935f) observed that certain abnormal cystic structures appeared just dorsal to the prostatic urethra or else in the neighborhood of the epididymis after prolonged treatment with estrone. He considered these structures to be hypertrophic Müllerian rudiments. Those near the epididymis may well have been the hydatids of Morgagni, such as were found by Zuckerman and Krohn (1937) to occur frequently in the monkey and to respond to estrone treatment. Further relevant researches have been carried out by Wells (1936) who found that, in the male ground squirrel, injection of estrogen during the period of low sexual activity produced gross hypertrophy of all accessory sexual organs, both in normal and castrated males. It produced a metaplastic stratification and cornification of the epithelium in the bulbar gland, Cowper's glands and prostatic ducts, and stratification with cornification occurred in the collecting tubules of the prostate gland. In this connection, the effect of estrogens on the uterus masculinus of the rabbit is of interest. This organ in the adult male is large and has a highly complex epithelium reminiscent of the endometrium of the pseudopregnant female. Castration causes retrogression both of the fibro-connective tissue and of the epithelium. Administration of estrogen to the immature or castrated male causes considerable size increase in the organ, but no development of the epithelium. Administration of large quantities of methyltesto-sterone, by contrast, caused full evolution of the epithelium.

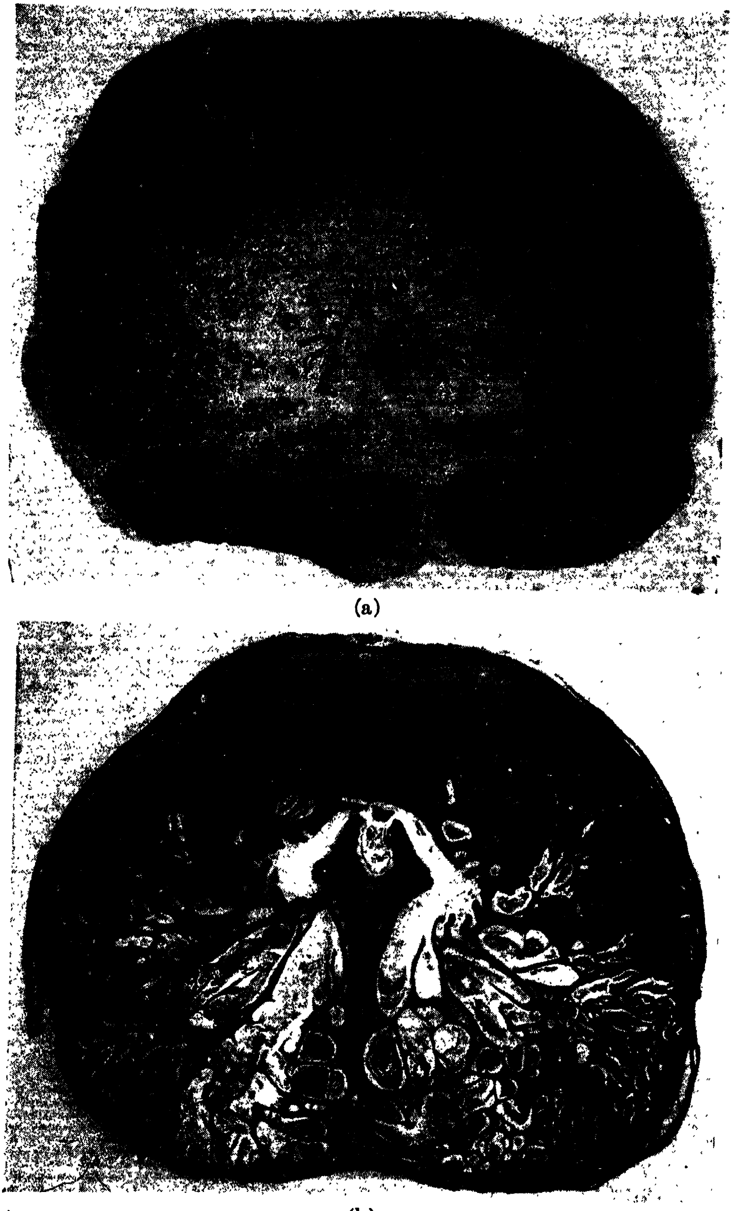
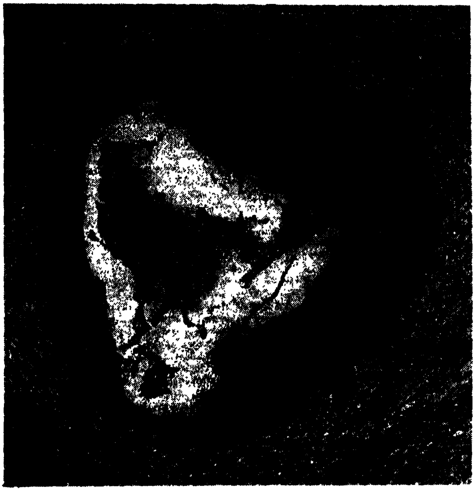
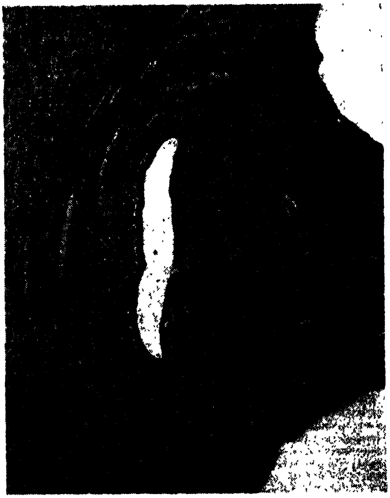


Fig. 6.—Effect of estrogen on the dog prostate. (a) Prostate of normal dog. (b) Prostate of dog after treatment with estrone, showing the cystic condition of the dilated and cornified glands. (From Zuckerman and Groome, 1937.)



(a)



(b)

Fig. 7.—Protection of the male monkey from the effects of estrone. (a) Uterus masculinus of immature rhesus monkey receiving 100 γ of estrone daily for 14 days, showing extensive cornification. (b) Uterus masculinus of immature rhesus monkey receiving daily 100 γ of estrone plus 5 mg. of androstanediol for 14 days, showing normal condition. (From Zuckerman and Parkes, 1936b.)

It appears, therefore, that the uterus masculinus of the rabbit has completely different hormonal relations from those seen in the other species investigated.

Raynaud (1937) found the cat to be very sensitive to the action of estradiol. Bi-weekly injections, commenced at birth and totaling less than 2 mg. over 3 weeks, produced in male kittens great hypertrophy of the muscular coats of the bladder and metaplasia and cornification of the epithelium in the neighborhood of its base. There was also cornification and desquamation of the urethral epithelium. These results were confirmed by Starkey and Leathem (1939).

There seems to be general agreement on the facts relating to the response of the epithelium of the male reproductive organs to estrogen, and the morphological basis of the response is clearly of the greatest interest. The interpretation put forward by Lacassagne and by de Jongh, and supported by Burrows, is that Müllerian elements enter into the structure of the tissues showing an epithelial response, though relevant embryological information for the rat and mouse is scarce or lacking. Zuckerman (1936a), however, pointed out that certain tissues of the male respond to estrone—the urethra of the rat (Burrows, 1934b) and monkey, for instance, which are definitely not related embryologically to the Müllerian ducts. He suggested that the epithelium of the urogenital sinus must be included with the epithelium of the Müllerian ducts, as tissues upon which estrogens have a specific action. In a more recent review, Zuckerman (1940) has examined the problem in considerable detail. He distinguishes between the estrogen-induced proliferation of cylindrical secretory cells, which is, in the main, confined to tissues derived from the Müllerian duct, such as the hydatids of Morgagni, and the proliferation, cornification and desquamation of stratified squamous epithelium, which may occur in tissues said to be derived from the Müllerian and Wolffian ducts, the urogenital sinus or the skin. Zuckerman concludes that the embryological components of the urogenital system intermix during development, at their points of contact and suggests that (in either sex) true Müllerian tissue responds to estrogens by glandular proliferation, and that epithelial metaplasia and stratification occurring in the reproductive tract in response to estrogenic stimulation may be regarded anatomically as a primary response of tissue in whose development estrogen-sensitive sinus epithelium has either played a direct or an indirect part. Burrows concluded that the specific action of estrogens on epithelia is confined to organs which (*a*) in some way serve the purpose of reproduction, or (*b*) are morphologically, though not otherwise, associated with reproduction, or (*c*) are embryological representatives of the reproductive system.

There has been much discussion of the relation between these changes in the male reproductive tract produced experimentally by injection of estrone, and those seen in benign enlargement of the human prostate. So far, however, no precise connection seems to have been demonstrated, and therapy based on the assumption of such a relation does not appear to have been particularly successful.

2. Mammary Gland

It is sometimes said that the mammary gland of the male can respond to the stimulus of estrogens as well as can that of the female. Astwood and Geschickter (1938), however, maintain that the mammary glands of male and female rats differ not only in structure but in their ability to respond to estrogenic stimuli, the difference being independent of the gonad and presumably genetic. Nevertheless, the male has been extensively used for studies of the effect of estrogens on the mammary glands, and much information is available about the behavior of the male gland rudiment (see citations by Nelson, 1936).

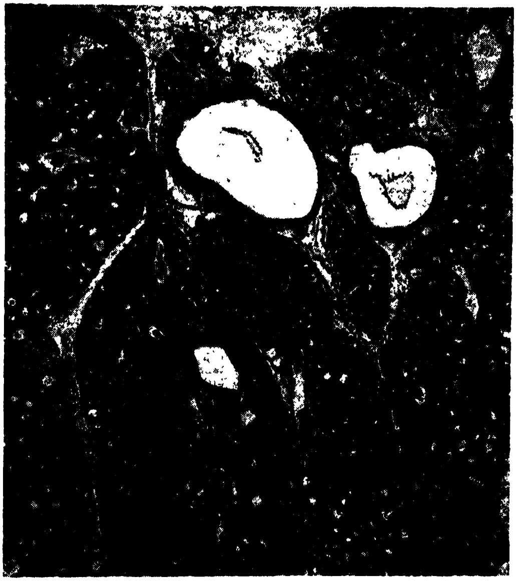


FIG. 8.—Effect of estrone on the mammary glands of the male guinea-pig. Mammary gland of male guinea-pig which absorbed 4 mg. of estrone from a tablet during 5 months, showing extensive alveolar and duct proliferation.

Attention had early been directed to the male guinea-pig by Steinach's successful ovarian transplants which, although containing no corpora lutea, were able to initiate full lactation. It is now clear that the mammary gland of the guinea-pig responds exceptionally vigorously to estrogenic hormones (Fig. 8). Haterius (1928) found ramification of the duct system and increase in size of the mammary lobules in males after treatment with estrogenic extracts, while Laqueur, Borchardt, Dingemanse, and de Jongh (1928) reported that similar treatment causes development of the mammary gland and teats equal to that seen in the pregnant female. Smelser (1933) studied in some detail the growth of the nipples of the estrogenized male guinea-pig, while Nelson and Pfiffner (1931) used estrogens and corpus luteum extracts combined. Subsequent workers such as Nelson and Smelser (1933), Nelson (1936,1937), Turner and Gomez (1934), and Lyons and Pencharz (1936), agree that estrogenic hormones will cause complete lobule-alveolar development in the male guinea-pig, which leads to lactation when injection of estrogen is stopped. Removal of the pituitary does not interfere with the glandular growth caused by estrogens in the male guinea-pig, but prevents the ensuing milk secretion (Nelson, 1935–36); Lyons and Pencharz, 1936). The action of the estrogen, therefore, appears to be a direct one on the gland, although milk secretion is dependent on the presence of the hypophysis.

The response to estrogens seems to be much less in other species. In male mice, Turner *et al.* (1932) were able to cause proliferation of the duct system and of prominent end buds, by prolonged treatment with estrone and estriol. Turner and Gomez (1934) studied the effect of estrogenic hormones on the mammary gland of male albino mice. They describe extensive duct development, but were unable to induce proliferation of the alveolar system even by prolonged treatment. Gardner (1935) reported that the injection of estrone or estrone benzoate into male mice caused proliferation of the mammary gland similar to that seen in the virgin female. Proliferation of the mammary ducts of male mice, accompanied by cystic mastopathy and hyperplasia of the epithelium, after over 50 days treatment with estrogens, is recorded by Burrows (1935c). In strains of mice which are prone to mammary cancer, the stimulation of the male gland by estrogens naturally leads to an increase in the incidence of the disease, as shown by Lacassagne (1932), Burrows (1935a), and Cramer and Horning (1936a).

Turner and Schultze (1931) found that there is considerable extension of the duct system, accompanied by a limited amount of alveolar proliferation in male rats given relatively high dosages of estrogen. Nelson (1935), however, observed considerable lobule-alveolar development in female rats after estrogenic hormone administration. Although there appears to be fairly general agreement that estrogens fail to stimulate mammary growth in hypophysectomized male rats (*cf.* Selye, Collip, and

Thomson, 1935; Reece, Turner, and Hill, 1936; Astwood, Geschickter, and Raush, 1937), a few reports to the contrary have appeared (Ruinen, 1932; Freud and de Jongh, 1935). Leonard (1943) has resolved the difficulty to some extent by investigations which showed that growth of the mammary gland end buds is stimulated in hypophysectomized immature male or female rats weighing less than 70 g. at operation if treatment is started immediately after the operation. No stimulation was seen if the rats weighed more (or much more) than 70 g., or if treatment was started 7 days after hypophysectomy. The observations of Reece and Leathem (1945), that the mammary glands of rats castrated and hypophysectomized at a body weight of 72–167 g. and injected with estradiol dipropionate from 5 days after the operation onwards did not respond, but that they did respond if lactogenic hormone was simultaneously given, further indicates that the pituitary gland plays a rôle in the response to estrogens. It is possible that, in Leonard's rats, enough residual pituitary hormone remained, or its effects remained, in rats injected before 7 post-operative days had elapsed, to enable the estrogen to stimulate mammary development.

Turner and de Moss (1934) could produce only duct proliferation by the injection of estrogens into normal male cats, and Turner and Gomez (1934) made similar observations on the dog. Houssay (1935) described considerable development of the gland and enlargement of the nipples in male dogs treated with up to 10,000 I.U. of estrone daily for 90 days. Turner and Frank (1932) observed duct development in the mammary glands of estrogenized male rabbits, associated with a limited amount of lobule proliferation similar to that found in the virgin female after prolonged estrus. The latter authors, and also Frazier and Mu (1935), record the secretion of milk in male rabbits treated for a long period with estrogenic hormone; this response seems to indicate some lobule development. Later work on the male rabbit has shown the development of ducts and acini following estrogenic stimulation, but indicates that full development is seen only if progesterone is given as well. Lyons and McGinty (1941) found that the best development of ducts and acini was obtained with one I.U. of progesterone daily in conjunction with 120 I.U. of estrone daily, and Scharf and Lyons (1941) also found the optimum combination for duct and alveolar development in immature male rabbits to be one I.U. of progesterone and 240 or more I.U. daily of estrone. In the mouse, Mixner and Turner (1942) record the inhibition of the action of progesterone in causing development of mammary acini by excessive doses of estrone. Gardner (1941) also records the inhibition of mammary growth in male mice by high doses of estrogens. Thus, the action of estrogen in causing mammary development would appear to depend on both the dosage level and on a balance with other substances.

Folley, Guthkelch, and Zuckerman (1939) found duct development in male rhesus monkeys treated for varying periods (6–484 days) with 0.7–290.4 mg. of estrone. Only one animal showed alveolar formation. de Fremery (1938) was unable to stimulate mammary development in a male goat by the inunction of amounts of estradiol benzoate which were effective in virgin females.

The earlier work on nipple growth in mammals was reviewed by Nelson (1936) who concluded that in the males (and spayed females) of all species studied, estrogen induces nipple growth. Small doses elicit a maximal response, thus, Lewis and Turner (1942) found that 0.1 γ daily of diethylstilbestrol induced nipple growth to $2\frac{1}{2}$ times the length of the controls in male guinea-pigs. In contradiction of the earlier results with the guinea-pig (*cf.* Lyons and Pencharz, 1936; Gomez and Turner, 1936), Samuels, Reinecke, and Petersen (1941) found that, in hypophysectomized rats, 1000 I.U. of estradiol benzoate given every other day for 30 days did not cause nipple growth.

VIII. Effects on the Adrenal Glands

It might be expected that estrogens would cause specific changes in the adrenals of the males of those species in which an ovary-dependent sex dimorphism of the adrenals exists, and perhaps more general changes by direct or indirect means in other species. The mouse and the rat have mostly been used in experiments of this kind. Castration of the immature male mouse causes the retention of the X-zone of the adrenal cortex, a zone which is normally present in the female until the first pregnancy.

Martin (1930) reported that the prolonged injection of estrin into castrated immature male mice, as into normal or spayed females, caused total degeneration of the X-zone, while untreated controls showed only incomplete atrophy. Burrows (1936d), using pure estrogens (estrone, estradiol, estriol, *etc.*), applied to the skin of males, found several different types of reaction:—(*a*) no obvious abnormality, (*b*) an increase in cortical thickness with the presence of an X-zone, (*c*) an increase in the vascular connective tissue between cortex and medulla, or (*d*) accumulation of cytoplasm and lipoid-like material between cortex and medulla. He suggests that the sequence of changes is the reappearance of the X-zone and the production of lipoid-like material by the cells of this zone.

Cramer and Horning (1937) produced "brown degeneration" of the adrenals of mice of both sexes by prolonged percutaneous administration of estrogens, and Lacassagne and Raynaud (1937) found that repeated estrin injections causes a like degeneration, with withdrawal of the "symplasmes phagacytaires." They found no influence on the development of the X-zone in female mice.

Cramer and Horning (1936b) state that an outstanding change in estrin-treated mice over a prolonged period is the complete disappearance of fat and a condition of cachexia. Danner (1938) finds that continued estrin (Progynon) administration to male mice results in degeneration of the pars fasculata accompanied by cachexia, and loss of hair, body fat and body weight. This author agrees with Burrows and with Deanesly (1939) in finding that, with large doses of estrone, the male mouse adrenal usually hypertrophies and a transitory development of the X-zone may take place; at a later stage the adrenals tend to resemble in size and shape those of postpubertal females in which the original X-zone has degenerated. After cessation of estrone treatment the adrenals diminish in size and gradually revert to their normal appearance (Deanesly, 1939). The varied results obtained in experiments of this type may be attributed to differences in the dosage employed and to the fact that estrone, in addition to having a direct action on the adrenal, may have an indirect one through the anterior pituitary body.

In the rat, in which the sex dimorphism consists in the smaller size of the adrenals of the male, castration causes an increase in the size of the male adrenal, a change which can be reversed or prevented by the administration of androgen (Section IX). Administration of estrogens to the normal male causes a definite increase in the size of the adrenal (Korenchevsky and Dennison, 1934a, 1935) produced chiefly by hypertrophy of the cortex and only slightly by that of the medulla. This change may, perhaps, be interpreted as a partial castration effect, since the treatment causes a very considerable drop in testis activity as judged by the size of the accessory reproductive glands. If, as seems likely, the castration effect is exerted through the pituitary body, the effect of estrone on the adrenals of the normal male rat may perhaps be due to a series of four events, namely, depression of the gonadotropic activity of the pituitary body, decrease in testis activity, increase in the adrenotropic activity of the pituitary body, and hypertrophy of the adrenal gland.

IX. INTERACTION OF ESTROGENIC AND ANDROGENIC SUBSTANCES

1. Protection of Males from the Effects of Estrogens

The supposition that the pathological changes caused by estrogens in the prostate of mice (Section VII) bear some relation to those which constitute benign enlargement of the prostate in senescent men suggested investigation of the effect of the simultaneous administration of androgens. A similar suggestion arose from the fact that in some experiments it had been noted that the pathological changes induced by estrogens were inhibited or prevented by the presence of functional testes. Weichert and Hale (1943), in particular, noticed that young male rats responded to estrogen more readily than adult males, and castrated males more readily than any intact ones. De Jongh (1935b) carried out some early experiments on the combined administration of androgens and estrogens, and was able to show that, when androgenic preparations of male urine were simultaneously injected with estrogen, the pathological changes in the prostate did not occur. These results were later extended and given in detail by de Jongh (1935a) who found that 3 units of androgen (=300 γ androsterone) would inhibit the effect on the prostatic epithelium of 1 γ of estrone, and by Korenchevsky and Dennison (1935) who found that the pathological changes produced in the seminal vesicles, prostate and coagulating glands by 18 γ of estrone could be prevented by the simultaneous injection of 1.5 "capon units" of urinary androgens. Moreover, they found a cooperative effect of these combined injections in restoring the histological picture towards normal. Korenchevsky and Dennison explained the absence of any significant histological changes in normal rats injected with 18 γ of estrone by supposing that the natural hormones secreted by the testis have a neutralizing effect. Further papers followed from de Jongh and his co-workers on the inhibitory effect of androgens on the various changes produced by estrone, in the fibrous tissue of the vasa deferentia, in the epithelium of the excretory ducts of the seminal vesicles and prostate and in the epithelium of the cranial prostate (coagulating gland). Waterman (1936) showed that crystalline androsterone or androstanediol would also inhibit the change caused by estrone in the prostate of mice and, less effectively, those caused in the seminal vesicles. Van der Woerd and de Jongh (1936) found that testosterone and dehydroandrosterone had the same power. These authors showed that 7 γ of testosterone, or 70 γ of androsterone would completely or nearly protect the prostate from the effects of 1.5 γ of estrone, while 210 γ of dehydroandrosterone partially inhibited the effects of the estrogen. Investigation by van der Woerd and de Jongh (1938) of the relative effectiveness of androsterone and testosterone in inhibiting the estrone effect on the male accessory organs showed that 3 γ of testosterone had a similar effect to that of 210 γ of androsterone. Methyl testosterone was also effective (Waterman, 1937). The effect of synthetic estrogen on rats can also be prevented by the simultaneous administration of testosterone (Weichert and Hale, 1943). The protective action of androgens extends also to the bony changes caused in male mice by estrogenization (Gardner and Pfeiffer, 1938b). According to Rusch (1937) testosterone, but not progesterone or dehydroandrosterone in the doses used, was effective in preventing epithelial metaplasia and fibro-muscular hypertrophy in estrogenized mice.

It has been recorded above that the most obvious change in the prostate region of the rhesus monkey, following estrogen treatment, is cornification of the uterus masculinus. Zuckerman and Parkes (1936b) found that this effect can readily be prevented by the simultaneous injection of androgenic substance; 5 mg. daily of androstanediol obliterated the effect of 100 γ of estrone (Fig. 7b). The external effect was not regularly inhibited by this dosage of androgen. Subsequently, Zuckerman (1936b) showed that the same inhibitory effect was exerted by testosterone, the propionate having a prolonged action. The effect of 1.4 mg. of estrone in causing cornification of the uterus masculinus and urethra, and growth of the fibrous tissue of the utricular bed and seminal vesicles was inhibited by a total dose of 10 mg. of the propionate. Five times the amount of androgen did not, however, entirely prevent the external changes caused by estrone.

In the guinea-pig, also, extensive cornification of the uterus masculinus is caused by estrone treatment (Courrier and Cohen-Solal, 1936; Laqueur, 1936). The response can be inhibited by androstanediol, especially when its effectiveness is increased by the simultaneous administration of X-substance. Similarly, the fibromyoepithelioma produced in the utricular bed of male guinea-pigs by prolonged estrogenization can be prevented by the simultaneous administration of testosterone and other anti-fibromatogenic substances (Lipschütz *et al.*, 1945).

In some of these experiments, those of Zuckerman (1936b) and Zuckerman and Parkes (1936b), for instance, it has been specifically noted that, whereas the androgen has prevented the action of the estrogen, the absence of the normal androgenic effect on the glands implies that the estrogen has also inhibited the effect of the androgen. It is most improbable, however, that this antagonistic action of the two types of substance is brought about by any form of chemical interaction. In ovariectomized mice, for instance, the cornifying effect of estrone is inhibited by testosterone, but so is that of *trans*-androstenediol (Emmens and Parkes, 1938) which is very closely related to testosterone. It must be supposed, therefore, that the inhibitory action of the androgens when given simultaneously with estrogens is due to a direct antagonistic influence in the tissues.

Wolfe and Hamilton (1937) have reported partial suppression of the action of estrone on the pituitaries of normal male rats by the simultaneous injection of testosterone propionate. The percentage of chromophobes did not increase, and the other histological and weight changes were not as marked in rats receiving 10 daily injections of 500 γ of testosterone propionate in addition to 500 γ of estrone as in those receiving the same dose of estrone alone.

Albert (1942) also noted that the enlargement of the pituitary and adrenals of male rats which followed the administration of 300 γ of α-estradiol per day was completely inhibited by the simultaneous administration of 10 mg. daily of testosterone. Although proliferation of the stroma was also prevented by testosterone, squamous metaplasia of the prostatic epithelium was not. Similar results were reported by Segaloff and Dunning (1945) who found that the enlargement of the pituitary gland of male rats caused by the administration of 50 γ of α-estradiol daily was prevented by the simultaneous administration of 0.5 mg. of testosterone per day.

The effects of various estrogens in feminizing the developing male rat embryo are said by Greene *et al.* (1941) to be completely inhibited by adequate doses of testosterone propionate; 14 mg. of testosterone propionate nullified the effects of 3.5 mg. of α-estradiol. Conversely, the masculinizing effects of androgens were inhibited by estrogens.

2. Synergistic Actions of Estrogens and Androgens

The difficulties attendant on a study of the cooperative actions of estrogens and androgens were noted in Section I. It seems to be the usual practice to describe the action of two substances as synergistic when the increase in weight of the test-object, resulting from the simultaneous administration of given amounts of each, is significantly greater than the added increases in weight produced by the separate administration of the same amounts to different animals of the same species. There is often considerable difficulty in demonstrating that either summation of effects or synergism has occurred, since extensive experimentation may be required to show that small differences in the mean weight of groups of test objects are consistent and significant.

Freud (1933), in describing the effects of estrone on the smooth muscle of the seminal vesicles, reported that the simultaneous administration of estrogenic and androgenic extracts to castrated rats caused a size increase in the seminal vesicles greater than could be accounted for by summation of the individual effects of the two types of substance if given separately. He referred to their synergistic action as "pace-making." Similar results were obtained by Korenchevsky and Dennison (1934b, 1935), Overholser and Nelson (1935) and de Jongh (1935a). Callow and Deanesly (1935) investigated the interaction between estrone and the first crystalline androgen available, androsterone. They came to the conclusion that the synergism, as measured by weight changes in the seminal vesicles of castrated rats, was slight and did little to answer the then important question, the cause of the relative ineffectiveness of pure androsterone on castrated rats. The cooperative action of various androgens with estrone or estradiol in castrated rats has been studied in detail by Korenchevsky and his co-workers. Estrone, in a dose of 6 γ per day, shows a cooperative effect on the action of 0.5–1.0 mg. of androsterone per day in causing growth of the seminal vesicles and prostate in castrated rats, but an antagonistic effect on the action of the androsterone on the pituitary and adrenal glands, which do not show regression from the castrate to the normal size. The cooperative effect of estrone with androsterone on the accessory sex organs can be replaced by increasing the dose of androsterone (Korenchevsky, Dennison, and Simpson, 1935). Androsterone with testosterone does not give even a summation effect (Korenchevsky, Dennison, and Brovsin, 1936).

Testosterone shows only a slight cooperative effect with estrone or estradiol (Korenchevsky *et al.*, 1936), that with estradiol being more marked in the dosage used (6 γ of estrone, or 20 γ of estradiol, 3 times a week, with 33 γ of testosterone daily for 23 days). Testosterone together with estrone or estradiol does not correct the hypertrophy of the adrenals and hypophysis of castrated rats. Testosterone and androstenedione, testosterone propionate and androstenediol have also been found to synergize when injected together, particularly on the seminal vesicles (Korenchevsky and Dennison, 1937; Korenchevsky, Dennison, and Hall, 1937). *trans*-Dehydroandrosterone does not show any definite synergism with estrone when injected daily in doses of 1 mg. and 6 γ, respectively (Korenchevsky and Dennison, 1936). Androstenedione or androstenediol and estrone also gave negative results except that the adrenals were not brought back to normal weight in the presence of estrone (Korenchevsky, Dennison, and Eldridge, 1937). In addition to the interactions already mentioned, Korenchevsky and his co-workers found that when estrogens are injected simultaneously with androgens, the gain in body weight and, in some cases, the fat deposition are decreased.

REFERENCES

1. Albert, S. 1942. *Endocrinology* **30,** 454.
2. Allanson, M. 1937. *Proc. Roy. Soc. London* **B124,** 196.
3. Astwood, E. B., and Geschickter, C. F. 1938. *Arch. Surg.* **36,** 672.
4. Astwood, E. B., Geschickter, C. F., and Raush, E. C. 1937. *Am. J. Anat.* **61,** 373.
5. Bachman, C., Collip, J. B., and Selye, H. 1935. *Proc. Roy. Soc. London* **B 117,** 16.
6. Barnes, B. O., Regan, J. F. W., and Nelson, W. O. 1933. *J. Am. Med. Assoc.* **101,** 926.
7. Beall, D. 1939. *Nature* **144,** 76.

8. Beall, D. 1940. *J. Endocrinol.* **2,** 81.
9. Bell, G. H., and Cuthbertson, D. P. 1943. *J. Endocrinol.* **3,** 308.
10. Berblinger, W. 1914. *Verhandl. deut. path. Ges.* **17,** 184.
11. Bialet-Laprida, Z. 1933a. *Compt. rend. soc. biol.* **114,** 727.
12. Bialet-Laprida, Z. 1933b. *Compt. rend. soc. biol.* **114,** 733.
13. Bonser, G. M. 1942. *J. Exptl. Biol.* **54,** 149.
14. Bonser, G. M., and Robson, J. M. 1940. *J. Path. Bact.* **51,** 9.
15. Borchardt, H., Dingemanse, E., de Jongh, S. E., and Laqueur, E. 1929. *Z. exptl. Med.* **68,** 86.
16. Bugbee, E. P., and Simond, A. 1926. *Endocrinology* **10,** 360.
17. Burch, J. C., and Cunningham, R. S. 1930. *Proc. Soc. Exptl. Biol. Med.* **27** 331.
18. Burns, R. K. 1939. *J. Morphol.* **65,** 497.
19. Burns, R. K. 1942. *Biol. Symposia* **9,** 125.
20. Burns, R. K., and Moore, C. H. 1941a. *Physiol. Zoöl.* **14,** 1.
21. Burns, R. K., and Moore, C. H. 1941b. *J. Urol.* **45,** 869.
22. Burrows, H. 1934a. *Brit. J. Surg.* **21,** 507.
23. Burrows, H. 1934b. *Nature* **134,** 570.
24. Burrows, H. 1935a. *Am. J. Cancer* **23,** 490.
25. Burrows, H. 1935b. *Am. J. Cancer* **24,** 613.
26. Burrows, H. 1935c. *Brit. J. Surg.* **23,** 191.
27. Burrows, H. 1935d. *J. Path. Bact.* **41,** 43, 218, 423.
28. Burrows, H. 1935e. *J. Physiol.* **85,** 159.
29. Burrows, H. 1935f. *Proc. Roy. Soc. London* **B118,** 485.
30. Burrows, H. 1936a. *Am. J. Cancer* **28,** 741.
31. Burrows, H. 1936b. *Brit. J. Surg.* **23,** 658.
32. Burrows, H. 1936c. *J. Path. Bact.* **42,** 161.
33. Burrows, H. 1936d. *J. Path. Bact.* **43,** 121.
34. Burrows, H. 1937. *J. Path. Bact.* **44,** 481, 699.
35. Burrows, H. 1945. Biological Actions of Sex Hormones. Cambridge Univ. Press, London.
36. Burrows, H., and Kennaway, N. M. 1934. *Am. J. Cancer* **20,** 47.
37. Callow, N. H., Callow, R. K., and Emmens, C. W. 1939. *J. Endocrinol.* **1,** 76.,
38. Callow, R. K., and Deanesly, R. 1935. *Biochem. J.* **29,** 1414.
39. Chang, M.-C. 1942. *J. Endocrinol.* **3,** 192.
40. Collip, J. B., Selye, H., and Thomson, D. L. 1933. *Proc. Soc. Exptl. Biol. Med.* **30,** 544.
41. Courrier, R. 1934. *Compt. rend. soc. biol.* **117,** 1117.
42. Courrier, R., and Cohen-Solal, G. 1936. *Compt. rend. soc. biol.* **121,** 903.
43. Courrier, R., and Gros, G. 1935a. *Compt. rend. soc. biol.* **118,** 683.
44. Courrier, R., and Gros, G. 1935b. *Compt. rend. soc. biol.* **118,** 686.
45. Cramer, W., and Horning, E. S. 1936a. *Lancet* **1,** 247.
46. Cramer, W., and Horning, E. S. 1936b. *Lancet* **1,** 775.
47. Cramer, W., and Horning, E. S. 1936c. *Lancet* **1,** 1056.
48. Cramer, W., and Horning, E. S. 1937. *J. Path. Bact.* **44,** 633.
49. Crooke, A. C., and Korenchevsky, V. *Proc. Roy. Soc. Med. London* **28,** 1266.
50. Danner, M. 1938. *Klin. Wochschr.* **17,** 658.
51. David, K., Freud, J., and de Jongh, S. E. 1938. *Biochem. J.* **28,** 1360.
52. Deanesly, R., *J. Endocrinol.* 1939. **1,** 36.
53. Deanesly, R., and Parkes, A. S. 1938. *Lancet* **2,** 606.

54. Deanesly, R., and Parkes, A. S. 1941. *J. Endocrinol.* **2,** 487.
55. de Fremery, P. 1938. *Arch. néerland. zool.* **3,** 48.
56. de Jongh, S. E. 1935a. *Arch. intern. pharmacodynamie* **50,** 348.
57. de Jongh, S. E. 1935b. *Arch. néerland. physiol.* **5,** 28.
58. de Jongh, S. E., and Kok, D. J. 1935. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **5,** 177.
59. de Jongh, S. E., and Laqueur, E. 1934. *Nederl. Tijdschr. Geneeskunde* **78,** 3030.
60. del Castillo, E. B., and Pinto, A. 1937. *Rev. soc. argent. biol.* **13,** 426.
61. Desclin, L. 1934. *Arch. biol.* **45,** 503.
62. Dingemanse, E., Borchardt, H., and Laqueur, E. 1937. *Biochem. J.* **31,** 500.
62a. Dodds, E. C. 1944. *Vitamins & Hormones* **2,** 353.
63. Döhrn, M., Hohlweg, W., and Schoeller, W. 1933. *Arch. exptl. Path.* **172,** 261.
64. Emmens, C. W. 1942. *J. Endocrinol.* **3,** 64.
65. Emmens, C. W., and Parkes, A. S. 1938. *J. Path. Bact.* **47,** 279.
66. Engel, E. T. 1934. *Klin. Wochschr.* **1,** 540.
67. Erdheim, J. 1935. *Fortschr. Röntgenstr.* **52,** 234.
68. Folley, S. J., Guthkelch, A. N., and Zuckerman, S. 1939. *Proc. Roy. Soc. London* **B126,** 469.
69. Frazier, C. N., and Mu, J. W. 1935. *Proc. Soc. Exptl. Biol. Med.* **32,** 997.
70. Freud, J. 1933. *Biochem. J.* **27,** 1438.
71. Freud, J., and de Jongh, S. E. 1935. *Acta Brevia Néerland. Physiol., Pharmacol. Microbiol.* **5,** 47.
72. Gaarenstroom, J. H., and Levie, L. H. 1939. *J. Endocrinol.* **1,** 420.
73. Gallagher, T. F., Petersen, D. H., Dorfman, R. I., Kenyon, A. T., and Koch, F. C. 1937. *J. Clin. Invest.* **16,** 695.
74. Gardner, W. U. 1935. *Proc. Soc. Exptl. Biol. Med.* **33,** 104.
75. Gardner, W. U. 1936. *Am. J. Anat.* **59,** 459.
76. Gardner, W. U. 1937. *Anat. Record* **68,** 339.
77. Gardner, W. U. 1941. *Endocrinology* **28,** 53.
78. Gardner, W. U., and De Vita, J. 1940. *Yale J. Biol. Med.* **13,** 213.
79. Gardner, W. U., and Pfeiffer, C. A. 1938a. *Proc. Soc. Exptl. Biol. Med.* **37,** 678.
80. Gardner, W. U., and Pfeiffer, C. A. 1938b. *Proc. Soc. Exptl. Biol. Med.* **38,** 599.
81. Gomez, E. T., and Turner, C. W. 1936. *Proc. Soc. Exptl. Biol. Med.* **34,** 320, 404.
82. Greene, R. R., Burrill, M. W., and Ivy, A. C. 1938. *Science* **88,** 130.
83. Greene, R. R., Burrill, M. W., and Ivy, A. C. 1939. *Proc. Soc. Exptl. Biol. Med.* **41,** 169.
84. Greene, R. R., Burrill, M. W., and Ivy, A. C. 1940. *Am. J. Anat.* **67,** 305.
85. Greene, R. R., Burrill, M. W., and Ivy, A. C. 1941. *J. Exptl. Zoöl.* **87,** 211.
86. Greene, R. R., Burrill, M. W., and Ivy, A. C. 1942. *Physiol. Zoöl.* **15,** 1.
87. Griffiths, M., and Young, F. G. 1942. *J. Endocrinol.* **3,** 96.
88. Halpern, S. R., and d'Amour, F. E. 1934. *Proc. Soc. Exptl. Bol. Med.* **32,** 108.
89. Hamilton, J. B., Heslin, J. E., and Gilbert, J. 1937. *J. Urol.* **37,** 725.
90. Harsh, R., Overholser, M. D., and Wells, L. J. 1939. *J. Endocrinol.* **1,** 261.
91. Haterius, H. O. 1928. *Proc. Soc. Exptl. Biol. Med.* **25,** 471.
92. Heckel, N. J., and Steinmetz, C. R. 1941a. *J. Clin. Endocrinol.* **1,** 664.
93. Heckel, N. J., and Steinmetz, C. R. 1941b. *J. Urol.* **46,** 319.
94. Hohlweg, W. 1934. *Klin. Wochschr.* **13,** 92.
95. Hohlweg, W., and Döhrn, M. 1931. *Wien. Arch. Med.* **21,** 426.

96. Hooker, C. W., and Pfeiffer, C. A. 1942. *Cancer Research* **2,** 759.

97. Hooker, C. W., and Pfeiffer, C. A. 1943. *Endocrinology* **32,** 69.

98. Houssay, B. A., 1935. *Compt. rend. soc. biol.* **120,** 502.

99. Huffman, M. N., MacCorquodale, D. W., Thayer, S. A., Doisy, E. A., Smith, G. V., and Smith, O. W. 1940. *J. Biol. Chem.* **134,** 591.

100. Huggins, C., and Clark, P. J. 1940. *J. Exptl. Med.* **72,** 747.

101. Koch, F. C. 1937–1938. *Harvey Lectures* 205.

102. Koneff, A. A., Simpson, M. E., and Evans, H. M. 1946. *Anat. Record* **94,** 169.

103. Korenchevsky, V., and Dennison, M. 1934a. *Biochem. J.* **28,** 1474.

104. Korenchevsky, V., and Dennison, M. 1934b. *Biochem. J.* **28,** 1486.

105. Korenchevsky, V., and Dennison, M. 1935. *J. Path. Bact.* **41,** 323.

106. Korenchevsky, V., and Dennison, M. 1936. *Biochem. J.* **30,** 1514.

107. Korenschevsky, V., and Dennison, M. 1937. *Biochem. J.* **31,** 862.

108. Korenchevsky, V., Dennison, M., and Brovsin, I. 1936. *Biochem. J.* **30,** 558.

109. Korenchevsky, V., Dennison, M., and Eldridge, M. 1937. *Biochem. J.* **31,** 467.

110. Korenchevsky, V., Dennison, M., and Hall, K. 1937. *Biochem. J.* **31,** 1434.

111. Korenschevsky, V., Dennison, M., and Simpson, S. L. 1935. *Biochem. J.* **29,** 2534.

112. Lacassagne, A. 1932. *Compt. rend.* **195,** 630.

113. Lacassagne, A. 1933. *Compt. rend. soc. biol.* **113,** 580.

114. Lacassagne, A. 1934a. *Compt. rend. soc. biol.* **115,** 469.

115. Lacassagne, A. 1934b. *Compt. rend. soc. biol.* **115,** 579.

116. Lacassagne, A. 1934c. *Compt. rend. soc. biol.* **116,** 95.

117. Lacassagne, A., and Raynaud, A. 1937. *Compt. rend. soc. biol.* **124,** 1183, 1186.

118. Lacassagne, A., and Villela, E. 1933. *Compt. rend. soc. biol.* **114,** 870.

119. Laqueur, E. 1936. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **6,** 9.

120. Laqueur, E., Borchardt, H., Dingemanse, E., and de Jongh, S. E. 1928. *Deut. med. Wochschr.* p. 12.

121. Laqueur, E., Dingemanse, E., Hart, P. C., and de Jongh, S. E. 1927. *Klin. Wochschr.* **6,** 1859.

122. Laqueur, E., and de Jongh, S. E. 1928. *J. Am. Med. Assoc.* **91,** 1169.

123. Leonard, S. L. 1943. *Endocrinology* **32,** 229.

124. Leonard, S. L., Meyer, R. K., and Hisaw, F. L. 1931. *Endocrinology* **15,** 17

125. Lewis, A. A., and Turner, C. W. 1942. *Endocrinology* **30,** 585.

126. Lipschütz, A. 1934. *Compt. rend. soc. biol.* **116,** 89.

127. Lipschütz, A. 1935. *Quart. J. Exptl. Physiol.* **25,** 109.

128. Lipschütz, A., Palacios, L., and Akel, J. 1936. *Compt. rend. soc. biol.* **121,** 205.

129. Lipschütz, A., Yanine, D., Schwarz, J., Bruzzone, S., Acuna, J., and Silberman, S. 1945. *Cancer Research* **5,** 515.

130. Lyons, W. R., and McGinty, D. A. 1941. *Proc. Soc. Exptl. Biol. Med.* **48,** 83.

131. Lyons, W. R., and Pencharz, R. I. 1936. *Proc. Soc. Exptl. Biol. Med.* **33,** 589.

132. Marrian, G. F. 1938. *Ergeb. Vitamin- u. Hormonforsch.* **1,** 419.

133. Martin, S. J. 1930. *Proc. Soc. Exptl. Biol. Med.* **28,** 41.

134. Matthews, C. S., Schwabe, E. L., and Emery, F. E. 1942. *Endocrinology* **30,** 89.

135. Mazer, C., Israel, S. L., and Alpers, B. J. 1936. *Endocrinology* **20,** 753.

136. Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J. 1930. *Proc. Soc. Exptl. Biol. Med.* **27,** 702.

137. Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J. 1932. *Endocrinology* **16,** 655.

138. Miller, E. W., Orr, J. W., and Pybus, F. C. 1943. *J. Path. Bact.* **55,** 137.
139. Mixner, J. P., and Turner, C. W. 1942. *Endocrinology* **30,** 591.
140. Montpellier, J., and Chiapponi, L. 1930. *Compt. rend. soc. biol.* **104,** 373.
141. Moore, C. R. 1944. *Am. Naturalist* **78,** 97.
142. Moore, C. R., and Price, D. 1930. *Proc. Soc. Exptl. Biol. Med.* **28,** 38.
143. Moore, C. R., and Price, D. 1932. *Am. J. Anat.* **50,** 13.
144. Moore, R. A., and McLellan, A. M. 1938. *J. Urol.* **40,** 641.
145. Mühlbock, O. 1940. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **10,** 69.
146. Mulligan, R. M. 1943. *Proc. Soc. Exptl. Biol. Med.* **54,** 21.
147. Nelson, W. O. 1933. *Anat. Record* **55,** 31.
148. Nelson, W. O. 1934. *Proc. Soc. Exptl. Biol. Med.* **32,** 452.
149. Nelson, W. O. 1935–36. *Proc. Soc. Exptl. Biol. Med.* **33,** 222.
150. Nelson, W. O. 1935. *Anat. Record* **64,** 52.
151. Nelson, W. O. 1936. *Physiol. Revs.* **16,** 488.
152. Nelson, W. O. 1937. *Am. J. Anat.* **60,** 341.
153. Nelson, W. O., and Pfiffner, J. J. 1931. *Anat. Record* **51,** 51.
154. Nelson, W. O., and Smelser, G. K. 1933. *Am. J. Physiol.* **103,** 374.
155. Noble, R. L. 1938. *Lancet* **2,** 192.
156. Overholser, M. D., and Nelson, W. O. 1935. *Anat. Record* **62,** 247.
157. Parkes, A. S., and Emmens, C. W. 1944. *Vitamins & Hormones* **2,** 361.
158. Parkes, A. S., and Zuckerman, S. 1935. *Lancet* **1,** 925.
159. Raynaud, A. 1937. *Compt. rend. soc. biol.* **127,** 215.
160. Raynaud, A. 1939a. *Compt. rend. soc. biol.* **130,** 872, 1012, 1061.
161. Raynaud, A. 1939b. *Compt. rend. soc. biol.* **131,** 218.
162. Reece, R. P., and Leathem, J. H. 1945. *Proc. Soc. Exptl. Biol. Med.* **59,** 122.
163. Reece, R. P., Turner, C. W., and Hill, R. T. 1936. *Proc. Soc. Exptl. Biol. Med.* **34,** 204.
164. Ruinen, F. H. 1932. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **2,** 161.
165. Rusch, H. P. 1937. *Endocrinology* **21,** 511.
166. Samuels, L. T., Reinecke, R. M., and Petersen, W. E. 1941. *Proc. Soc. Exptl. Biol. Med.* **46,** 379.
167. Scharf, G., and Lyons, W. R. 1941. *Proc. Soc. Exptl. Biol. Med.* **48,** 86.
168. Schoeller, W., and Gehrke, M. 1933. *Biochem. Z.* **264,** 352.
169. Segaloff, A., and Dunning, W. F. 1945. *Endocrinology* **36,** 238.
170. Selye, H., Collip, J. B., and Thomson, D. L. 1935. *Proc. Soc. Exptl. Biol. Med.* **32,** 1377.
171. Severinghaus, A. E. 1934a. *Anat. Record* **60,** 43.
172. Severinghaus, A. E. 1934b. *Proc. Soc. Exptl. Biol. Med.* **31,** 593.
173. Severinghaus, A. E. 1937. *Physiol. Revs.* **17,** 556.
174. Sharpey-Schafer, E. P., and Zuckerman, S. 1940. *J. Endocrinol.* **2,** 431.
175. Shumacker, H. B., and Lamont, A. 1935. *Proc. Soc. Exptl. Biol. Med.* **32,** 1568.
176. Smelser, G. K. 1933. *Physiol. Zool.* **6,** 395.
177. Smith, O. W. 1944. *Endocrinology* **35,** 146.
178. Spencer, J., d'Amour, F. E., and Gustavson, R. G. 1931. *Proc. Soc. Exptl. Biol. Med.* **28,** 500.
179. Spencer, J., d'Amour, F. E., and Gustavson, R. G. 1932. *Am. J. Anat.* **50,** 129.
180. Starkey, W. F., and Leathem, J. H. 1939. *Anat. Record* **75,** 85.

181. Steinach, E., and Kuhn, H. 1926. *Biol. Generalis* **2,** 815.
182. Tausk, M., and de Fremery, P. 1935. *Acta Brevia Néerland. Physiol., Pharmacol. Microbiol.* **5,** 19.
183. Turner, C. W., and de Moss, W. R. 1934. *Mo. Agr. Expt. Sta. Res. Bull.* **207.**
184. Turner, C. W., and Frank, A. H. 1932. *Mo. Agr. Expt. Sta. Res. Bull.* **174,** 29.
185. Turner, C. W., Frank, A. H., Gardner, W. U., Schultze, A. B., and Gomez, E. T. 1932. *Anat. Record* **53,** 227.
186. Turner, C. W., and Gomez, E. T. 1934. *Mo. Agr. Expt. Sta. Res. Bull.* **207,** 28–29.
187. Turner, C. W., and Schultze, A. B. 1931. *Mo. Agr. Expt. Sta. Res. Bull.* **157.**
188. van der Woerd, L. A. 1938. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **8,** 110.
189. van der Woerd, L. A., and de Jongh, S. E. 1936. *Acta Brevia Néerl. Physiol, Pharmacol. Microbiol.* **6,** 88.
190. van der Woerd, L. A., and de Jongh, S. E. 1938. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **8,** 80.
191. van Wagenen, G. 1935. *Anat. Record* **63,** 387.
192. Vasquez-Lopez, E. 1944. *J. Path. Bact.* **46,** 1.
193. Wade, N. J., and Doisy, E. A. 1935. *Endocrinology* **19,** 77.
194. Wallace, C. 1946. Cambridge University.
195. Waterman, L. 1936. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **6,** 56.
196. Waterman, L. 1937. *Acta Brevia Néerland. Physiol. Pharmacol. Microbiol.* **7,** 121.
197. Weichert, C. K., and Hale, H. B. 1943. *Endocrinology* **33,** 16.
198. Weichert, C. K., and Kerrigan, S. 1942. *Endocrinology* **30,** 741.
199. Wells, L. T. 1936. *Anat. Record* **64,** 475.
200. Westerfeld, W. W. 1942. *J. Biol. Chem.* **143,** 177.
201. Wolfe, J. M., and Hamilton, J. B. 1937. *Endocrinology* **21,** 603.
202. Wolfe, J. M., and Phelps, D. 1935. *Proc. Soc. Exptl. Biol. Med.* **32,** 1305.
203. Wolfe, J. M., and Wright, A. W. 1938. *Endocrinology* **23,** 200.
204. Zondek, B. 1934. *Nature* **133,** 209, 494.
205. Zondek, B. 1935. *Schweiz. med. Wochschr.* **65,** 1168.
206. Zondek, B. 1936a. *Lancet* **1,** 10.
207. Zondek, B. 1936b. *Lancet* **1,** 776.
208. Zondek, B. 1937. *Folia Clin. Orientalia* **1,** 1.
209. Zuckerman, S. 1936a. *Lancet* **1,** 135.
210. Zuckerman, S. 1936b. *Lancet* **1,** 1259.
211. Zuckerman, S. 1938. *J. Anat.* **72,** 264.
212. Zuckerman, S. 1940. *Biol. Revs.* **15,** 231.
213. Zuckerman, S., and Groome, J. R. 1937. *J. Path. Bact.* **44,** 113.
214. Zuckerman, S., and Krohn, P. L. 1937. *Phil. Trans. Roy. Soc. London* **228,** 147.
215. Zuckerman, S., and Parkes, A. S. 1936a. *J. Anat.* **70,** 323.
216. Zuckerman, S., and Parkes, A. S. 1936b. *Lancet* **1,** 242.
217. Zuckerman, S., and van Wagenen, G. 1935. *J. Anat.* **69,** 497.

The Biology of Antithyroid Agents

BY HARRY A. CHARIPPER AND ALBERT S. GORDON

CONTENTS

Page

I. Introduction 274
II. Historical Survey 275
III. Effects on the Gross and Microscopic Structure of the Thyroid and Pituitary Glands 277
  1. Mammals 277
    a. Thyroid Changes. 277
    b. Reversibility of Effect. 279
    c. Pituitary Changes 279
  2. Birds 280
  3. Reptiles. 281
  4. Amphibians 281
  5. Fishes. 282
  6. Invertebrates. 283
  7. During Ontogeny. 283
IV. Effects on Body Growth and General Metabolism. 283
  1. Mammals 283
  2. Birds 285
  3. Amphibians 286
  4. Fishes. 286
V. Mechanism of Action 287
  1. Effects on Iodine Metabolism. 287
    a. *In Vivo* Experiments 287
    b. *In Vitro* Experiments. 289
  2. Effects on Enzyme Systems 290
    a. Peroxidase. 290
    b. Cytochrome Oxidase 291
    c. Succinoxidase 291
    d. Xanthine Oxidase 291
    e. Phosphatase. 291
    f. Tyrosinase. 292
    g. Proteolytic Activity 292
    h. Oxidation-Reduction Potentials and Enzyme Activity 292
  3. Effects on the Pituitary Thyrotropin[1] Mechanism 293
  4. Direct Effects on the Thyroid 295
VI. Effects on Organs Other than the Thyroid and Pituitary 295
  1. Adrenal Gland 295
  2. Reproductive Organs and Accessories 296
  3. Liver and Kidney. 297
  4. Peripheral Blood and Bone Marrow. 297
  5. Other Organs. 298
VII. Factors Influencing Activity of Antithyroid Agents 299
  1. Age. 299
  2. Sex. 299

---

[1] See footnote 2, page 233.

Page
3. Diet 300
4. Temperature 300
5. Method of Administration 300
6. Species Differences 301
VIII. Metabolism of Antithyroid Agents 301
IX. Chemical Nature of Antithyroid Agents 303
X. Clinical Applications 305
XI. Conclusion 307
References 307
Addendum 315

I. INTRODUCTION

The thyroid gland, in the course of regulating the processes of metabolism, growth and differentiation within the organism, sensitively reflects changes in both the external and internal environment. Among the factors found to exert an influence on the size, gross and microscopic structure, and physiology of the thyroid have been diet, age, sex, heredity, psychic and idiosyncratic conditions, hygienic state, season, temperature, radiation, barometric and oxygen pressures, humidity, endocrine factors and deficiencies, blood, immune and antihormone principles, and certain types of chemicals and drugs. In view of the rather widespread occurrence of hyperthyroidism, research extending over many years has sought to uncover agencies by which it would be possible safely and effectively to depress thyroid activity. The classical experiments of Coindet (quoted by Means, 1937) and those of Plummer (1923) have served to establish and stimulate further research on the importance of inorganic iodine as a rational treatment for various types of goiter.

Within the last few years, a series of researches, involving the joint efforts of the morphologist, physiologist, biological and organic chemists, and the clinician, have led to the recognition and profitable utilization of certain antithyroid chemicals. The treatment of laboratory animals and man with such compounds has not only provided new insight as to the possible etiology and therapy of the hyperthyroid syndrome but has also yielded valuable new information concerning the mechanism of action of the thyroid gland and its relation to other endocrine glands, particularly the pituitary. It is not practicable in a review of this length to include information on all types of antithyroid agents. The literature abounds with information on the role of iodine in thyroid function, and several good discussions are available (McCarrison, 1917, 1933, 1937; Means, 1937; Salter, 1940). The important subject of the relation of vitamins to thyroid function has been well covered by Drill (1943), while the interrelationship between thyroid, carbohydrate metabolism and diabetes was reviewed in the preceding volume of this series by Houssay

(1946). This report will be concerned largely with a survey and analysis of the work on the more recently investigated chemical agents affecting thyroid function.

II. HISTORICAL SURVEY

Although the presence and importance of goitrogenic agents in various types of food such as beef and liver (McCarrison, 1917) had been realized for many years, the experiments by Webster and his group at Johns Hopkins served to intensify interest in this problem. In their first study, Chesney, Clawson, and Webster (1928) observed greatly enlarged thyroids in rabbits fed a diet containing cabbage leaves. The glands were hyperemic, possessed columnar epithelia and contained little colloid. This hyperplastic condition was associated with a lowered heat production (Webster, Clawson, and Chesney, 1928). The administration of iodine tended to cause involution of the gland and to augment the metabolic rate (Webster and Chesney, 1928; Webster, 1929). These workers were unable to explain this effect. Marine, Baumann and Cipra (1929), however, attributed it to the presence of a powerful goitrogenic agent in cabbage, and in addition demonstrated that other members of the *Brassica* group such as Brussel sprouts and cauliflower were also effective; fresh or steamed carrots and lettuce did not produce this action. Since Hoffman (quoted by Marine *et al.*, 1932) had detected nitriles in *Cruciferae,* it was suggested by Marine and his coworkers (1932) that some cyanide compound might be the causative factor. Their experiments revealed that certain nitriles, particularly acetonitrile, were effective goitrogenic agents in the rabbit. Aromatic nitriles possessed little potency and NaSCN was ineffective. Thiocyanates, however, have been found more recently to exert goitrogenic activity in rats (Astwood, 1943b; Rawson, Tannheimer, and Peacock, 1944) and human beings (Barker, Lindberg, and Wald, 1941; Foulger and Rose, 1943; Rawson, Hertz, and Means, 1943). The explanation offered for the goitrogenic action was that certain cyanide compounds, by depressing general tissue oxidation, create a greater demand for thyroid principle which, in turn, increases the gland's need for iodine. To meet these conditions the thyroid undergoes compensatory enlargement.

The existence of goitrogens in soybeans was shown by McCarrison (1933) who observed thyroid enlargement in young rats fed a soybean diet. Confirmation of these results was given by Sharpless (1938) who found glands as large as 5 times normal in rats given a diet containing unprocessed soybean flour. It was demonstrated by Sharpless, Pearsons, and Prato (1939) that the minimal iodine requirement for thyroid function in the rat was increased 100% as a result of this diet and that, as for cabbage, the thyroid enlargement could be prevented by iodine. Certain extraction procedures led them to the conclusion that the active agent was probably a cyanogen. It is interesting to note that even larger thyroids (8–10 times normal) may be produced with soybean diets in the chick (Wilgus, Gassner, Patton, and Gustavson, 1941), which appears to be a more sensitive test object for certain goitrogenic agents than any other species as yet examined. More recently (Borland, 1943) goiters have also been induced in the tadpole with cabbage or methyl cyanide.

Significant contributions to the problem of goitrogenicity and diet have been made by Purves and his collaborators in New Zealand. The conclusion was reached (Hercus and Purves, 1936; Kennedy and Purves, 1941) that goitrogenic activity is a property shared by the seeds of many *Brassica* species including swede, soft and hard turnip, chou moellier and rape. Kennedy and Purves (1941) observed that, although a large dose of iodine considerably inhibited the gross thyroid enlargement induced by a rape seed diet, the hyperplastic condition of the gland was not affected. Later Purves (1943) found that the thyroid hyperplasia caused by rape seed could be inhibited by thyroxine in doses as small as 3 γ/100 g. body weight daily. On the other hand, iodide and diiodotyrosine, even in large doses, were unable to overcome completely the rape seed effect. This lack of response to iodine represents an often mentioned point of difference in the behavior of the goitrogens described above (*i.e.*, *Brassica* leaves, soybeans, and cyanide or cyanide-like compounds) and that of certain *Brassica* seeds, including rape.

The importance of the pituitary in the thyroid response to *Brassica* seeds was demonstrated by the New Zealand group (Griesbach, 1941; Griesbach, Kennedy, and Purves, 1941). Kennedy (1942) concluded that the goitrogenic agent in rape seed was a derivative of urea. Oral administration of allylthiourea, in fact, led to marked hypertrophy and hyperplasia of the thyroid gland, and the histological appearance of both the thyroid and pituitary glands was identical with that in rats fed rape seed. Previously Richter and Clisby (1941), in studying the effects of phenylthiourea on graying of hair in rats, noted that this compound also induced marked thyroid hyperplasia. The Mackenzies reported the thyroid-enlarging effects of thiourea in 1942.

The discovery that the sulfonamides are powerful goitrogenic agents was made by Mackenzie, Mackenzie, and McCollum (1941). In examining the influence of sulfaguanidine on nutrient synthesis by intestinal bacteria they noted that this compound produced marked enlargement of the thyroid. As with the rape seed diet and thiourea, the glands displayed marked hyperemia and hyperplasia. Sulfadiazine, sulfapyridine and sulfanilamide also induced thyroid overgrowth, whereas sulfanilic acid and guanidine carbonate were found to be ineffective (the Mackenzies, 1942). The Mackenzies (1943a) and Astwood and his coworkers (1943) confirmed and extended the previous findings that thiourea and certain aminobenzene compounds are effective goitrogenic agents. Both groups of investigators observed that the thyroid enlargement was accompanied by a decrease in BMR[2] and could not be overcome by the administration of iodine but could be prevented by thyroid substance or by pituitary ablation. These two latter reports, and that of Astwood (1943a) demonstrating the marked activity of thiouracil, have aroused such unusual interest in the antithyroid agents that they have become, within the last 4 years, one of the most actively investigated subjects in the entire field of endocrinology.

III. EFFECTS ON THE GROSS AND MICROSCOPIC STRUCTURE OF THE THYROID AND PITUITARY GLANDS

1. *Mammals*

a. Thyroid Changes. The feeding of thiourea and several of its derivatives has revealed their goitrogenic activity in most species of mammals. This includes the rat (Richter and Clisby, 1941; the Mackenzies, 1942, 1943a; Astwood, Sullivan, Bissell, and Tyslowitz, 1943), rabbit (Baumann, Metzger and Marine, 1944; Wagner-Jauregg and Schreiber, 1944), mouse and dog (the Mackenzies, 1943a), goat (Schultze and Turner, 1945), cattle (Schultze and Turner, 1945), monkey (Engle and Aranow, 1946) and man (see clinical references below). The guinea pig thyroid has been reported as being refractory to sulfapyridine and sulfaguanidine in doses capable of inducing thyroid enlargement in other species (the Mackenzies, 1943a). Thiouracil, however, appears to be an effective goitrogenic agent in the guinea pig (Williams, Weinglass, and Kay, 1944). Engle and Aranow (1946) find the monkey to be relatively refractory to thiouracil, but with greater than usual doses administered for longer periods of time, the typical hyperplastic response eventually occurs.

The feeding of rape seed (Kennedy and Purves, 1941), aminobenzene (the Mackenzies, 1943a, 1944; Gordon, Goldsmith, and Charipper, 1945b) or thiourea compounds (Astwood, Sullivan, Bissell, and Tyslowitz, 1943; Astwood and Bissell, 1944; Gordon, Goldsmith, and Charipper, 1945b) to rats usually induces most rapid thyroid enlargement during the second and third weeks. Following this, the growth rate decreases, but slight further enlargement continues for periods as long as 8 weeks. The thyroid growth is symmetrical and is usually in direct proportion to the concentration of goitrogen administered and the level of the agent in the blood (the Mackenzies, 1943a; Higgins, 1945). Thyroxine is more effective than iodine in overcoming the goitrogenic activity of the thioureas and aminobenzene compounds (the Mackenzies, 1943a; Astwood, Sullivan, Bissell, and Tyslowitz, 1943). Iodine may actually potentiate the effects of sulfaguanidine (the Mackenzies, 1943a) and thiouracil (Williams, Weinglass, and Kay, 1944) on the thyroid gland.

---

[2] Basal Metabolic Rate.

Most of the histological changes have been described for the rat (Kennedy and Purves, 1941; the Mackenzies, 1943a; Astwood, Sullivan, Bissell, and Tyslowitz, 1943; Paschkis, Cantarow, Rakoff, and Rothenberg, 1945) and these will serve as an illustration for the mammalian species in general. With optimal doses of effective agents, first signs are visible as early as 24–48 hours and include hyperemia, heightening of the follicular epithelium and loss of colloid. By the end of the first week, only shreds of colloid usually remain and the epithelium is transformed from the normal, low cuboidal type to high cuboidal or columnar possessing large vesicular nuclei; basophilic alterations of the acinar cell cytoplasm also occur at this time. After about 2 weeks, the numbers and sizes of the individual follicles have increased, loss of colloid is virtually complete and the epithelium is almost exclusively of the columnar type. By this time the hypertrophy and hyperplasia have become so extensive as to produce crowding and invagination of the acinar epithelium. Papillary growths appear which project into the colloid-depleted lumina and give rise to new follicles by a process of budding; in many areas cord-like masses of cells proliferate among the follicles eventually giving the gland a compact tubular appearance. This intensely active histological picture may be maintained for periods as long as 100 days (the Mackenzies, 1943a). Diminution of the thyroid hyperplasia, however, occurs with the rape seed diet after about 8 weeks (Griesbach, 1941). A similar regression has been reported in the human being treated with thiouracil (Reveno, 1945). It would be of interest to determine whether this thyroid exhaustion is due to pituitary failure, to a reduced sensitivity of the thyroid, or to an increased destruction or elimination of the goitrogenic agent. For the first few months of treatment, signs of scarring, necrosis or neoplasia are not usually encountered. Desquamation of follicular cells, however, has been observed occasionally with phenylthiourea (Richter and Clisby, 1941), sulfapyridine (Astwood, Sullivan, Bissell, and Tyslowitz, 1943) and sulfaguanidine (the Mackenzies, 1944). In the monkey (Engle and Aranow, 1946) prolonged treatment with thiouracil results in the formation of peculiar colloid-containing vesicles within the hyperplastic follicles.

Continuous administration of the rape seed diet to rats for excessively long periods of time (8–27 months) may produce thyroid adenomata

(Griesbach, Kennedy, and Purves, 1945). These bodies are usually discrete and sometimes multiple; their follicles are enlarged, irregular, contain some colloid, and display tall columnar cells. The epithelium is narrower than that seen in the normal hyperplastic gland and possesses more darkly staining nuclei. In some cases, fusion of adjoining adenomata occurs, forming large cystic areas. Addition of KI, in amounts sufficient to bring the daily intake of iodine to 4 γ, tends to delay, but not to prevent, these abnormal growths. Thyroxine (10 γ/day) does not result in the disappearance of these bodies but causes a flattening of the epithelium and a reaccumulation of dense colloid. Similar benign tumorous growths have been produced in the rat with combined or alternate treatment with allylthiourea and the carcinogen, 2-acetylaminofluorene (Bielschowsky, 1944, 1945). Structures resembling adenocarcinomas have appeared within the thyroid adenomata of 2 animals treated continuously with thiourea for approximately 2 years, and in one animal, distinctly visible pulmonary metastases of thyroid tissue were apparent (Purves and Griesbach, unpublished). Dalton, Morris, and Dubnik (1946) have also recently reported the appearance of neoplastic thyroid growths in abnormal sites in C3H strain mice given prolonged thiouracil treatment. Greater use will probably be made of goitrogenic agents as a technique in elucidating the etiology and development of malignant thyroid growths.

b. Reversibility of Effect. Reaccumulation of colloid, decrease in vascularity and size of the follicles with a reversion of the epithelium to the cuboidal type occurs soon after discontinuation of sulfaguanidine (the Mackenzies, 1943a; Astwood, Sullivan, Bissell, and Tyslowitz, 1943), or thiourea (Astwood, Sullivan, Bissell, and Tyslowitz, 1943) in the rat and after interruption of thiouracil treatment in the monkey (Engle and Aranow, 1946). With promizole (Higgins, 1945) the restoration of the normal pattern is somewhat delayed.

The return of thyroid size and weight to normal levels is usually comparatively slow (Astwood, Sullivan, Bissell, and Tyslowitz, 1943; the Mackenzies, 1944). Goldsmith, Gordon, and Charipper (1945b) still found enlarged thyroids 65 days after discontinuation of thiourea treatment in rats which had been fed the drug for 161 days.

c. Pituitary Changes. Sharpless and Hopson (1940) were among the first to report changes in the numbers of basophilic and acidophilic cells of the anterior lobes of rats fed a soybean diet. These alterations could be only partially corrected by iodine but completely overcome with thyroid substance. Griesbach (1941) has provided a detailed account of the pituitary changes in rats fed a rape seed diet. Within 14 days, decreases in the numbers of acidophils and degranulation of these elements occur accompanied by an hypertrophy and increase in the numbers of the basophilic cells; mitotic figures within the basophils are frequently encountered. Hyalinization of the cytoplasm of the enlarged basophils is seen by the second to third week. Clear vacuoles within the hyalin matter make their appearance by the fourth week. By 56 days the basophilic picture has attained its greatest prominence and acidophils have almost completely disappeared. Large "thyroidectomy" or "signet-ring" cells are visible at this time. The Golgi apparatus within these enlarged cells has become greatly hypertrophied and is of the basophil type. From the eighth week of treatment on, there is a slow steady return of the histological picture to the normal condition. By the eighth month, basophils have decreased significantly in size and number, fewer show hyalinized cytoplasm, and acidophilic elements have reappeared in large numbers. These alterations in pituitary structure appear to be well correlated with the hyperplasia and subsequent regression of the thyroid cellular elements in rats fed the rape seed diet. Although Kennedy (1942) obtained quite similar results with thiourea, it was not stated whether he also observed retrogressive changes after the eighth week. The progressive increase in basophilia and decrease in acidophilia have also been found with sulfaguanidine (the Mackenzies, 1943a, 1944), thiourea and sulfathiazole (Leblond and Hoff, 1944), thiouracil (Salter, Cortell, and McKay, 1945) and *p*-aminobenzoic acid (Gordon, Goldsmith, and Charipper, 1945b) in the rat, and in the chronically thiouracilized thyrotoxic patient (Reveno, 1945). These alterations in pituitary cytology can be rectified by the administration of small quantities of thyroxine (Griesbach and Purves, 1943, 1945). It is difficult to explain the inability of Williams *et al.* (1944) to observe such changes with thiouracil in the rat.

No marked alterations in pituitary weight attend treatment of the rat with goitrogenic agents. Although Higgins (1945) has reported slight increases in pituitary weight following the feeding of the sulfonamide, promizole, these changes were not correlated with the increases in thyroid weight. Moreover, no significant changes in hypophyseal weight in rats have been found following treatment with thiouracil (Williams, Weinglass, Bissell, and Peters, 1944) or thiourea (Leathem, 1945a).

2. Birds

Mixner, Reineke, and Turner (1944) have studied the effects of thiourea and thiouracil on the thyroid gland of the chick. Maximal enlargement occurred with thiouracil in a concentration of 0.1% when tested over a 2-week period. They also noted that this thyroid hypertrophy could be prevented by small doses of thyroxine. Astwood, Bissell, and Hughes (1944) observed the marked effectiveness of several thiourcylenes on the chick thyroid. After 10 weeks with thiouracil, the most active compound tested, the weights of the glands averaged 45 times greater than those of the controls, a value considerably larger than that attained with any dosage in the mammal. Since the chick thyroid shows a more pronounced response to thiourea compounds, it would be of interest to determine the effects of long continued treatment with such agents in this species with the view of determining the possible incidence and nature of neoplastic growths. More extensive experiments in several species of fowl have been recently reported (Schultze and Turner, 1945; Andrews and Schnetzler, 1946; VanderLaan and Bissell, 1946a) with substantially the same results. The extent of thyroid enlargement is proportional to the concentration of agent administered up to a certain point, beyond which no further growth (VanderLaan and Bissell, 1946a) or even smaller sized glands (Astwood, Bissell, and Hughes, 1944; Schultze and Turner, 1944; 1945) are produced. Anatomical changes occur after 5 days of administration of thiouracil to the chick (Larson, Keating, Peacock, and Rawson, 1945a). Rape seed is not as effective as thiouracil on the chick thyroid (Turner, 1946).

The Mackenzies (1943a) reported the thyroids of the chick and the mature bird to be refractory to treatment with 2–3% sulfaguanidine for periods as long as 30 days. They attributed this to a species difference and suggested the possibility of the conversion of the active sulfonamide to the inactive acetylated form within the body of the bird. Similar failure of thyroid response has been obtained with sulfadiazine and *p*-aminobenzoic acid in the chick (Astwood, Bissell, and Hughes, 1944). More recently, however, VanderLaan and Bissell (1946a) have reported slight, but significant enlargement of the thyroid with 4-aminobenzoic acid, sulfadiazine and sulfaguanidine in this animal. The cause of this discrepancy in result is not clear.

3. Reptiles

No reports have thus far appeared. The alleged dependence of such reactions as moulting on thyroid activity (Noble and Bradley, 1933; Schaefer, 1933) would make an investigation of the response of this group to the goitrogenic agents worthwhile.

4. Amphibians

Structural changes in the thyroid gland and inhibition of metamorphosis have been reported for *Rana pipiens* tadpoles immersed in solutions of thiourea (Gordon, Goldsmith, and Charipper, 1943). Hughes and Astwood (1944a) have found that thiouracil can prevent thyrotropic hormone from stimulating metamorphosis in *Rana clamitans.* Thiourea and thiouracil also inhibit metamorphosis in *Rana sylvatica* (Lynn and De Marie, 1946). The changes in thyroid size and histology induced with thiourea have been observed, in general, to be similar to those reported in the mammal (Gordon, Goldsmith, and Charipper, 1945c). Enlarged glands showing increased numbers of follicles, columnar acinar epithelium, scanty colloid, and marked hyperemia are seen in larvae treated for 3–7 weeks. The regression in thyroid size and histological activity, which occurs after 3–4 months of treatment, has been shown to be due, most likely, to pituitary failure (Gordon, Goldsmith, and Charipper, 1945c). As with the chick (the Mackenzies, 1943a; Astwood, Bissell, and Hughes, 1944), sulfadiazine and *p*-aminobenzoic acid are inactive; both fail to inhibit the effect of thyrotropic hormone on metamorphosis in *Rana.* It would appear that the tadpole thyroid exhibits a more delayed response to thiourea and is more readily damaged than the mammalian gland (Gordon, Goldsmith, and Charipper, 1945c). *Triturus viridescens* has been found by Adams (1946b) to be relatively refractory to thiourea, high concentrations acting over a long period of time being necessary to produce thyroid enlargement. Singer (quoted by Astwood, 1944–45) has found no effect of thiourea on moulting in this form.

5. *Fishes*

The effects of thiourea on the fish thyroid were first described by Goldsmith, Nigrelli *et al.* (1944). Immersion of several species in thiourea solutions results in thyroid enlargement after 73 days; these glands also show the marked hyperplasia seen in the mammal. Prolonged treatment of fish with this drug may cause proliferation of thyroid tissue into abnormal sites (Nigrelli, personal communication) as in the case for the rat (Griesbach, Kennedy, and Purves, 1945; Bielschowsky, 1945; Dalton, Morris, and Dubnik, 1946).

Studies should be made of the effects of goitrogenic agents on pituitary structure in organisms lower than the mammal. The well acknowledged difficulty of surgical thyroidectomy in most lower vertebrates makes the use of antithyroid drugs for "chemical thyroidectomy" a most valuable tool to investigate such problems as the time in ontogeny or phylogeny when "thyroidectomy" cells make their appearance, and to determine whether thyrotropin production and release in these lower forms can be correlated with any special pituitary cell type, as has been done for the mammal (Griesbach and Purves, 1943, 1945).

6. *Invertebrates*

Thiourea is markedly toxic when fed to insect larvae. Experiments are required to determine whether the inhibition of development induced in the house-fly (McGovran and Piquett, 1943) and *Drosophila* (Goldsmith and Harnly, 1946) is a specific response to thiourea compounds or whether it merely represents a hypersensitivity of insect tissue to the toxic effects of this general chemical grouping. The inhibitory influence of thiourea on the development of the sea urchin (Bevelander, 1946) requires similar clarification.

7. *During Ontogeny*

Recent work (Goldsmith, Gordon, and Charipper, 1944; Hughes, 1944; Williams and Kay, 1944; Goldsmith, Gordon, and Charipper, 1945b) has pointed to the placental transmission of goitrogens, since the feeding of thiourea or thiouracil to pregnant rats causes enlargement and hyperplasia of the fetal thyroids. In addition, the retardation of the growth of the suckling young as a result of continued treatment of the mothers (Hughes, 1944; Goldsmith, Gordon, and Charipper, 1945b) strongly suggests the mammary passage of these agents. Williams, Kay, and Jandorf (1944) have actually detected 3 times as mũch thiouracil in the milk than in the blood of patients receiving thiouracil. Similarly, in the fowl, thiouracil feeding for 3 weeks results in the appearance of the drug in the egg (Andrews and Schnetzler, 1945). Egg size, production and hatchability, however, are not affected by the drug. Human young born to hyperthyroid mothers, treated with moderate doses of thiouracil during pregnancy, also are quite normal and display no visible goiters (Williams, 1946). Whether these differences represent a higher resistance of bird and human tissue to the drug or whether it is due to the presence of different amounts of the goitrogen in the embryonic tissues of the different species, remains to be established. The fetal goat thyroid is unaffected before the mid-gestational period when thiourea or thiouracil is fed to the mothers (Schultze and Turner, 1945). However, after this time, the fetal glands display considerable enlargement and hyperplasia. This may indicate the particular period of fetal development at which the pituitary-thyroid mechanism begins to function (Schultze and Turner, 1945).

IV. EFFECTS ON BODY GROWTH AND GENERAL METABOLISM

1. *Mammals*

Decreased food intake and inhibition of growth in young rats given diets containing 2% sulfaguanidine were reported by Astwood and his collaborators (1943). Reduction in BMR was not apparent during the first two weeks of treatment, but by the fourth week, the oxygen consumption had diminished approximately 30%. Neither growth nor food intake was impaired in hypophysectomized rats given similar treatment. Using this same drug, the Mackenzies (1943a, 1944) detected a reduction in oxygen consumption as early as the first week in normal rats. A 30% drop in BMR, attained by the fourth week, was maintained as long as 140 days, and the withdrawal of the drug from the diet resulted in a return of the BMR to normal levels within two weeks. Retardation of growth, lessened food intake, and decreased BMR have also been observed in rats fed thiourea (Leathem, 1945a, 1945b) or thiouracil (Williams, Weinglass, Bissell, and Peters, 1944; Gordon, Goldsmith, and Charipper, 1946). These changes paralleled, in general, those occurring in surgically thyroidectomized rats (Meyer and Ransom, 1945; Gordon, Goldsmith, and Charipper, 1946). Barker (1945a) demonstrated that the increase in BMR following thyrotropin injections was considerably smaller in the thiouracil-treated than in control rats. Although Hartzell (1945) found no inhibition of growth in rats fed thiourea for three years, he pointed out that the highest dose employed was insufficient to evoke thyroid enlargement. At 0.5% levels, however, this drug caused a 30% reduction in BMR within two weeks (Goldsmith, Gordon, and Charipper, 1945a). Continued feeding of 3% *p*-aminobenzoic acid to rats resulted in reductions in BMR as marked, and within the same time interval, as those occurring with 0.2% thiouracil (Gordon, Goldsmith, and Charipper, 1945b). The decrease in BMR produced in rats with promizole (Higgins, 1945) or thiourea and methylthiouracil (Christensen, 1945) was found to be directly proportional to the extent of thyroid hyperplasia and loss of colloid. Administration of goitrogens to thyroidectomized (the Mackenzies, 1943a) or hypophysectomized (Higgins and Ingle, 1946) rats has been reported to produce no greater reductions in BMR than those brought about by removal of these organs alone. However, Dempsey and Astwood (1943) have found that thiouracil administered continuously to rats for periods of 2–3 months may induce a depression in BMR greater than that detected in surgically thyroidectomized animals. This finding has been interpreted to mean that such drugs can inhibit both the synthesis of thyroxin within the thyroid and that which is claimed to occur in tissues other than this gland (Chapman, 1941; Morton, Chaikoff *et al.*, 1943).

The observations on growth and metabolism described above indicate quite conclusively that the goitrogenic thioureylene and aminobenzene compounds produce effects similar to those occurring after thyroid ablation. Further support for this statement is found in the fact that thyroid substance prevents or reverses most of these changes. In addition, cretinous changes (*e.g.*, lessened growth, impaired development and delayed ossification) take place in rats subjected to pre- and postpartum treatment with thiouracil (Hughes, 1944) or thiourea (Goldsmith, Gordon, and Charipper, 1945b; unpublished). Also, increased fat deposition occurs in swine fed thiouracil (Muhrer and Hogan, 1945) and thiourea reduces milk output in goats and cattle (Schultze and Turner, 1945), as does surgical thyroidectomy. Other observations fortifying this conclusion include: (1) alterations in heart function and conduction time during treatment with thiourea (Leblond and Hoff, 1944; Waller and Charipper, 1945), thiouracil (Leblond and Hoff, 1944; Mann, 1945) or sulfathiazole (Leblond and Hoff, 1944); (2) changes in plasma protein constituents with thiourea (Leathem, 1945a, 1945b) or thiouracil (Moore, Levin, and Smelser, 1945); (3) effects on kidney, adrenal and heart weights (Leblond and Hoff, 1944); and (4) correction of BMR, salt, nitrogen, cholesterol and carbohydrate metabolism derangements in hyperthyroid patients (Astwood, 1943a; McGavack, Gerl *et al.* 1944; Paschkis, 1944; Sloan and Shorr, 1944; Williams, Bissell *et al.*, 1944; Barr and Shorr, 1945). Relatively small amounts of thiouracil (0.1%) fed to rats from the 21st day of life may actually stimulate growth processes, and this has been interpreted as due to the beneficial action on growth of a slightly hypothyroid state (Astwood, 1944–45). The alterations in metabolism ordinarily induced by goitrogenic substances do not develop in dogs (Danowski, Man, and Winkler, 1946) or monkeys (Aranow, Engle, and Sperry, 1946) subjected to thiourea or thiouracil treatment. Yet such agents will eventually evoke the typical hyperplastic response in the thyroid glands of the dog (the Mackenzies, 1943a, 1944). In the monkey, it should be remembered that even after surgical thyroidectomy no marked hypothyroid symptoms develop (Jailer, Sperry *et al.*, 1944; Sperry, Jailer, and Engle, 1944). It has been indicated also that the maintenance of a normal metabolism in the dog is relatively independent of thyroxine (Danowski, Man, and Winkler, 1946). In addition, dog thyroids form much smaller amounts of thyroid hormone *in vitro* than do rat glands (Morton and Chaikoff, 1943).

2. Birds

Concentrations of 0.1–0.2% thiouracil in the diet have been variously reported as causing no change (Astwood, Bissell, and Hughes, 1944), inhibiting (Kempster and Turner, 1945), or increasing (Schnetzler, Andrews, and Hauge, 1945) normal body weight gains in fowl. These discrepancies may be due perhaps to the differences in the ages of the birds and lengths of treatment employed. Most observers agree, however, that fat deposition is increased (Schultze and Turner, 1944; Andrews and Schnetzler, 1946) as a result of the administration of this drug. Unfortunately, no experiments on the BMR of fowl under treatment with these agents have been performed. Higher dosages of thiouracil (0.5%) produce such cretinous changes as marked inhibition of body growth and impairment of bone, muscle, and feather pattern development (Astwood, Bissell, and Hughes, 1944; Juhn, 1944, 1946; Domm and Blivaiss, 1944, 1946). The bone deformities (perosis) induced in growing chickens with thiouracil are not prevented by manganese, choline, nicotinic acid, biotin or riboflavin (Briggs and Lillie, 1946); thyroxine was not tried.

3. Amphibians

Although developmental processes in tadpoles can be inhibited by thiourea (Gordon, Goldsmith, and Charipper, 1943, 1945c; Lynn and De Marie, 1946) and thiouracil (Hughes and Astwood, 1944a; Lynn and De Marie, 1946), growth continues unimpaired and actually becomes excessive. Giant tadpoles showing practically no advance of limb growth and delayed ossification processes are produced as a result of sustained thiourea treatment (Gordon, Goldsmith, and Charipper, 1945c). These changes are similar to those induced by thyroidectomy (Allen, 1918; Terry, 1918; Hoskins and Hoskins, 1919). Small quantities of thyroxine quickly check the inhibitory effects of thiourea (Gordon, Goldsmith, and Charipper, 1945c). Cessation of drug treatment results in a resumption of metamorphosis and in a relatively short time frogs, considerably larger than normal, emerge (Gordon, Goldsmith, and Charipper, 1945c).

4. Fishes

Growth and development of secondary sex characters in fish are arrested by thiourea treatment (Goldsmith, Nigrelli *et al.*, 1944). Thyroid substance will completely antagonize this action of thiourea (Nigrelli, Goldsmith, and Charipper, 1946).

Several of the effects developing with goitrogenic agents have not been typical of thyroidectomy. For example, an *increase* in liver weight following use of sulfonamides and thiourea (Leblond and Hoff, 1944; May, Mosely, and Forbes, 1946) has been observed. The increase in liver glycogen concentration noted in such livers (Hueper and Ichniowski, 1944; May, Mosely, and Forbes, 1946) has been attributed to lowered glycogenolysis resulting from inhibition of epinephrine action (Friedenwald and Buschke, 1943; May, Mosely, and Forbes, 1946). The changes in serum albumin reported with thiourea (Leathem, 1946) or thiouracil (Moore, Levin, and Smelser, 1945) also do not occur after thyroid removal. In addition, whereas thyroidectomy results in oliguria, thiouracil feeding induces a diuresis (Gordon, Goldsmith, and Charipper, 1946). The slower decline in food intake, water consumption, BMR, and cardiac rate with thiouracil as compared to thyroid removal in the rat has been attributed to the presence of stored hormone in the thyroid gland (Meyer and Ransom, 1945; Gordon, Goldsmith, and Charipper, 1946) the dissipation of which requires about five days (Astwood, 1944–1945).

By and large, however, in most species examined, the administration of the thiourea and sulfonamide compounds has induced metabolic changes and external symptoms similar to those attending the removal of the thyroid gland.

V. Mechanism of Action

1. *Effects on Iodine Metabolism*

As previously mentioned, goiters produced by nitriles and thiocyanates can be prevented or overcome by the administration of physiological quantities of inorganic iodine (Webster and Chesney, 1928; Webster, 1929; Sharpless, Pearsons, and Prato, 1939; Wilgus, Gassner *et al.*, 1941; Rawson, Hertz, and Means, 1943). In contrast, treatment with iodine, in doses as great as several thousand times the normal requirement, exerts little or no effect on the thyroid enlargement and hyperplasia induced by large quantities of thiourea and the aminobenzene compounds (the Mackenzies, 1943a; Astwood, Sullivan *et al.*, 1943; Baumann, Metzger, and Marine, 1944). Nevertheless, these latter agents have been found to exert a profound effect on iodine metabolism in the thyroid gland.

a. In Vivo Experiments. Loss of both inorganic and organic iodine represents perhaps the first manifestation of thyroid response to the administration of the goitrogens (Gersh and Baker, 1943; Baumann, Metzger, and Marine, 1944; Astwood and Bissell, 1944; Astwood, Bissell, and Hughes, 1944). Two days after an injection of thiourea, urinary excretion of iodine increases approximately 100% (Baumann, Metzger, and Marine, 1944). The protein-bound iodine and plasma iodine levels are markedly reduced in patients treated with thiouracil (Lowenstein, Bruger *et al.*, 1945) and rats given KSCN (Wolff, Chaikoff *et al.*, 1946). Thiouracil or sulfaguanidine administration to young rats is accompanied by a complete loss of iodine within five days (Astwood and Bissell, 1944). Removal of these drugs from the diet results in a reappearance of iodine within 24 hours (Astwood and Bissell, 1944). A possible clue as to the mechanism of this effect is afforded by the observation that extirpation of the pituitary or injection of thyroxine will prevent this reaccumulation of iodine within the gland (Astwood and Bissell, 1944). Similarly, thyrotropin injections retard the loss of thyroidal iodine in thiouracil-treated rats (Astwood and Bissell, 1944).

Not only do the goitrogens accelerate the loss of stored iodine from the thyroid but they also appear to inhibit the uptake or retention of administered iodine by the gland. Impairment of the ability of the thyroid to collect or retain radioactive iodine has been reported with thiourea (Keston, Goldsmith *et al.*, 1944; Couceiro and Vieira, 1944; Chagas, De Robertis, and Couceiro, 1945) thiouracil (Rawson, Tannheimer, and Peacock, 1944; Rawson, Cortell *et al.* 1944; Rawson, Evans *et al.*, 1944; Couceiro, Vieira, and de Moraes, 1944; Salter, Cortell, and McKay, 1945) and promizole (Higgins and Ingle, 1946). As early as one hour after injection of thiouracil and related compounds into rats or chicks the ability of the thyroid to accept radioactive iodine is considerably depressed (Larson, Keating, Peacock, and Rawson, 1945b; Rawson, McGinty, and Peacock, 1946). Withdrawal of the drug is followed by a rapid restoration of the iodine-concentrating capacity, and to a greater extent than in the controls (Larson, Keating, Peacock, and Rawson, 1945a, 1945b). In this connection, it is significant to note that thyrotropin injections will increase the uptake of radioactive iodine by the thyroid gland (Chagas, De Robertis, and Couceiro, 1945; Keating, Rawson *et al.* 1945).

Goiters caused by thiocyanate compounds appear to collect more iodine than do normal glands (Rawson, Tannheimer, and Peacock, 1944; Rawson, Cortell *et al.* 1944). Whereas patients under thiouracil treatment excrete most of the radioactive iodine administered (Rawson, Evans *et al.*, 1944; Rawson, Moore *et al.*, 1945) those under the influence of KSCN retain most of it (Rawson, Hertz, and Means, 1943; Rawson, Cortell *et al.*, 1944). Iodine abolishes the KSCN-induced goiter and renders the thyroidal iodine metabolism normal (Rawson, Hertz, and Means, 1943). According to Wolff *et al.* (1946), KSCN disappears rapidly from the blood following its administration to rats. In addition, they have shown that the uptake of radioactive iodine by the thyroid glands of KSCN-treated rats is consistently depressed, provided a high concentration of the goitrogen is maintained in the blood. When the KSCN in the blood has fallen to low levels (*i.e.*, between feedings or injections), the ability of the thyroid to collect iodine is restored. This work emphasizes that the activity of a goitrogen is not only determined by the inherent effectiveness of its chemical grouping but also by those factors which tend to produce a high level of the agent in the blood.

The decrease in iodine concentration of the thyroid gland in animals under the influence of thiouracil and related compounds occurs only when the iodine intake is relatively small. If large doses are administered, iodine collects in the gland (Astwood, 1944–5; Salter, Cortell, and McKay, 1945; VanderLaan and Bissell, 1946a; McGinty and Sharp, 1946) and to a degree proportional to the amount of iodine given (McGinty and Sharp, 1946). This iodine accumulation occurs despite the continued administration of the goitrogen and has little or no influence on the thyroid enlargement and hyperplasia (Astwood, 1944–5; Salter, Cortell, and McKay, 1945; McGinty and Sharp, 1946). The iodine taken up under these conditions remains water-soluble (Astwood, 1944–5) and appears to be only loosely bound to protein (Salter, Cortell, and McKay, 1945; McGinty and Sharp, 1946).

b. In Vitro Experiments. The employment of radioactive iodine has proved valuable in elucidating the mechanism of goitrogenic action. The extensive experiments of Chaikoff and his collaborators have definitely established the inhibitory effect of thiourea and aminobenzene compounds on thyroid hormone formation. Franklin and Chaikoff (1943) were the first to demonstrate the inhibitory action of sulfanilamide on the formation of diiodotyrosine and thyroxine from radioactive iodine by thyroid slices. Several other sulfonamides (Franklin and Chaikoff, 1944) and thiourea (Keston, Goldsmith *et al.*, 1944) were found to have a similar action. The list of active compounds was extended to include thiouracil, allylthiourea, *p*-aminobenzoic acid, *p*-aminophenylacetic acid, KSCN, NaCN, sodium azide and sulphide (Franklin, Chaikoff, and Lerner, 1944; Schachner, Franklin, and Chaikoff, 1944; Wolff, Chaikoff *et al.*, 1946). Washing thiourea-treated thyroid slices several times with Ringer's solution to remove all traces of the drug restores its ability to form organically linked iodine (Keston, Goldsmith, Gordon, and Charipper, unpublished).

The inhibitory action of thiourea, thiouracil, PABA,[3] sodium azide and sulfanilamide on the *in vitro* conversion of inorganic to organic iodine cannot be attributed to the absence of iodine in the tissue, since these agents exert no influence on the penetration of radioactive iodine into surviving thyroid slices (Franklin, Chaikoff, and Lerner, 1944; Schachner, Franklin, and Chaikoff, 1944; Keston *et al.* unpublished). KSCN, NaCN and sulphide, on the other hand, inhibit *both* the uptake and conversion of radioactive iodine by thyroid tissue *in vitro*. Thus, with the use of the goitrogenic agents it is possible to dissociate two mechanisms within the thyroid, one for concentrating iodine and another, which is independent of it, concerned with the synthesis of thyroid hormone.

The reports that the thioureas and sulfonamides depress the iodine-trapping mechanism of the thyroid gland *in vivo* but exert no influence on

[3] *p*-Aminobenzoic acid.

iodine uptake by isolated thyroid tissue are seemingly difficult to reconcile. VanderLaan and Bissell (1946b), however, have demonstrated that the thyroid gland of the drug-treated rat has the ability to collect considerable amounts of iodine but that the capacity of the gland to *retain* this iodine is lost within a few hours. The failure to detect iodine in the thyroid glands obtained from animals under treatment with these goitrogens may, therefore, be traced to the fact that the analyses have been carried out, invariably, 24–48 hours following administration of iodine, a time at which the iodine-collecting ability of the affected gland is no longer apparent. It thus appears that the antithyroid activity of the thioureas and sulfonamides may not be due to an effect exerted on iodine collection by the thyroid but rather to the ability of such compounds to prevent the retention of iodine and the chemical incorporation of this element into the thyroxine molecule. The inability of the thyroid, *in situ*, to retain iodine could, therefore, be simply the result of the failure of this element to become linked organically within the gland.

Conclusions about the operation of a goitrogenic agent within the body are not necessarily valid when drawn on the basis of its action on isolated thyroid tissue. It has been shown, for example, that certain sulfanilamide-like compounds, which are without effect on thyroid size or histology in the intact organism, markedly inhibit incorporation of inorganic iodide into diiodotyrosine and thyroxine *in vitro* (Taurog, Chaikoff, and Franklin, 1945). The possibility of conversion or inactivation, within the body, of a chemical grouping theoretically deduced to be goitrogenic, should always be considered.

2. Effects on Enzyme Systems

a. Peroxidase. Although peroxidase has not as yet been isolated from thyroid tissue (Glock, 1944), it has been shown to exist in the acinar epithelial cells of rat thyroid by the benzidine and other histochemical reactions (Dempsey, 1944; De Robertis and Grasso, 1946). Thiouracil (Dempsey, 1944; Williams, 1944a) and thiourea (De Robertis and Grasso, 1946) inhibit peroxidase activity in the follicular cells of the thyroid. Peroxidase has been shown to liberate free iodine from iodide and to catalyze the formation of a quinol ether linkage from *p*-cresol (Westerfeld and Lowe, 1942), a reaction analogous to the production of thyroxine from the etheric condensation of diiodotyrosine. Inhibition of such an enzyme system would, therefore, thwart two of the most important presently recognized steps in the synthesis of thyroid hormone (Johnson and Tewkesbury, 1942; Williams, 1944a; Harington, 1944; Astwood, 1944–5). It is of interest that sulfonamides do not inhibit peroxidase activity in thyroid tissue (De Robertis and Grasso, 1946).

b. Cytochrome Oxidase. The participation of the cytochrome-cytochrome oxidase systems in the biological oxidations of phenols and amines establishes it as another possible mechanism in the synthesis of the thyroid hormone (*cf.* the discussion of the mechanism of thyroxine formation in the recent review of Reineke, 1946). On the basis of certain *in vitro* experiments dealing with the inhibitory effects of CN, azide, sulphide and CO, Schachner, Franklin, and Chaikoff (1943) have asserted that the cytochrome-cytochrome oxidase system exerts its influence on the manufacture of both diiodotyrosine and thyroxin by thyroid tissue. Using histochemical techniques Dempsey (1944) demonstrated the presence of cytochrome oxidase in thyroid follicular cells but found that this enzyme was not inhibited by thiouracil. Simiarly, since sulfonamides, *p*-amino aromatic acids, thiourea, thiouracil or allylthiourea had no effect on the oxygen consumption of isolated sheep thyroid, it was concluded that such agents do not operate by interfering with the cytochrome oxidase system in the thyroid gland (Lerner and Chaikoff, 1945). In fact, the thyroids of rats fed thiouracil have been found to consume more oxygen than normal glands (Jandorf and Williams, 1944). The failure of thiouracil, certain sulfonamides and cysteine to inhibit cytochrome oxidase of thyroid tissue has also been observed by McShan *et al.* (1946). On the other hand, it has been reported (Paschkis, Cantarow, Rakoff, and Tillson, 1945b; Paschkis, Cantarow, and Tillson, 1945, 1946) that thiouracil and certain sulfonamides, but not *p*-aminobenzoic acid, depress the cytochrome oxidase system of rat thyroid both *in vivo* and *in vitro.* The effect, however, is not specific, since glutathione, cysteine and ascorbic acid are also inhibitory (Paschkis, Cantarow, and Tillson, 1946). The reason for these contradictory results is as yet not clear.

c. Succinoxidase. Succinic dehydrogenase exists in rat thyroid tissue but is not inhibited by sulfonamides, thiourea, thiouracil, cysteine or glutathione (McShan, Meyer, and Johansson, 1946).

d. Xanthine Oxidase. Keston (1944) has indicated that the formation of organically bound iodine in a milk medium may be catalyzed by the xanthine oxidase system and that this reaction is inhibited by thiourea. Although this suggests a possible participation of the flavoprotein system in biological iodinations, the importance of this enzyme in thyroid metabolism has still to be demonstrated. Moreover, Jandorf (quoted by Williams, 1944a) has obtained no inhibition of xanthine oxidase or triosephosphate-dehydrogenase with thiouracil.

e. Phosphatase. Recent histochemical analyses (Dempsey and Singer, 1946) have revealed the presence of both acid and alkaline glycerophosphatase in the follicles of rat thyroid. Experiments should be performed to determine the effects of the various antithyroid drugs on the activity of phosphatase in the gland in view of the recently demonstrated relation of this enzyme to thyroid basophilia (Dempsey and Singer, 1946). The report that glucose-1-phosphatase and fructose diphosphatase are concentrated in thyroid capillary endothelium is also of importance and brings to mind the suggestion of Thomas (1944) that thiourea may act by depressing secretion of thyroid hormone through an alteration of membrane permeability to precursor substances. If thiourea could be demonstrated to inhibit such phosphatase activity, experimental support for this theory would be available.

f. Tyrosinase. Inhibition of melanin formation in a tyrosine-tyrosinase system has been observed (Paschkis, Cantarow, Hart, and Rakoff, 1944) with thiourea compounds, sulfonamides and *p*-aminobenzoic acid. It is questionable as to whether this reaction has any significance in thyroid tissue because of the lack of correlation between goitrogenic and antityrosinase activity of certain compounds tested (Paschkis, Cantarow, Hart, and Rakoff, 1944).

g. Proteolytic Activity. There exists in the thyroid gland a proteolytic enzyme system concerned with the hydrolysis of colloid protein and the eventual reabsorption of the physiologically active thyroid products (Salter and Lerman, 1936; De Robertis, 1941; Gersh and Baker, 1943; Dziemian, 1943; De Robertis and Nowinski, 1945). Administration of thyrotrophin or sulfaguanidine to normal rats causes a decrease in the concentration of total protein of the colloid which can be correlated with an increase in proteolytic activity (Gersh and Baker, 1943). It is possible, therefore, that the rapid decrease in thyroidal iodine induced by the sulfonamides is due, in part, to their action on the proteolytic enzymes in the gland; this effect is most likely mediated through the pituitary thyrotropin mechanism. Thiourea, however, has been reported to exert no effect on the proteolytic mechanism within the thyroid (De Robertis and Grasso, 1946).

h. Oxidation-Reduction Potentials and Enzyme Activity. The aerobic redox potential of the follicular cells of the normal thyroid is higher than that of the colloid (De Robertis and Gonçalves, 1945). Activation of the gland by cold or thyrotropin injections elevates the value of the potential of the colloid to that of the cell. Thiourea is said to lower the potential of both the cytoplasm and the colloid of such activated glands (De Robertis and Gonçalves, 1945). This action has been related to the enzyme system behavior in the thyroid gland (De Robertis and Grasso, 1946).

It seems safe to conclude that a number of the goitrogenic drugs exert an inhibitory influence on certain of the enzyme systems within the thyroid concerned with the synthesis of thyroid hormone, and this effect may well represent one phase of the antithyroid action of such agents. The possibility, however, cannot be excluded that the antithyroid drugs may, in addition, express their effects through mechanisms other than enzymatic ones. For example, it has been suggested (Campbell, Landgrebe, and Morgan, 1944; Astwood, 1944–5) that the thioureas may operate through their ability to reduce iodine to iodide (Miller, Roblin, and Astwood, 1945) thus preventing the first step towards its incorporation into the thyroxine molecule. But, as Astwood has stated (1944–5), cysteine and glutathione can also effect this reduction but are without antithyroid action. Moreover, such an interpretation does not explain the activity of the goitrogenic sulfonamides which do not react with iodine *in vitro* (Miller, Roblin, and Astwood, 1945). A direct inactivation of the thyroid hormone has also been claimed (Dietrich and Beutner, 1944) because of the reduced ability of thyroxine to protect mice against acetonitrile in the presence of thiourea or thiouracil. Using the same methods, however, Malkiel (1946) obtained diametrically opposed results with thiouracil and sulfaguanidine. The observation that thyroxine augments the BMR to approximately the same levels in untreated thyroidectomized and sulfaguanidine-fed thyroidectomized rats (the Mackenzies, 1944) would tend to argue against any inactivation or destruction of thyroid principle by this group of goitrogens. György *et al.* (1943) have suggested that the antithyroid action of thiouracil and related compounds may simply be a reflection of the antioxygenic activity of the contained SH radical in its tautomeric form. Support for this idea is derived from the experiments of Taurog *et al.* (1945) who found a correlation between the ease with which certain goitrogenic agents become oxidized and their capacity to inhibit the *in vitro* formation of thyroxine. Paschkis, Cantarow, Hart, and Rakoff (1944) have remarked, however, that such a theory is unable to explain why such compounds as glutathione, with a greater SH content than thiouracil, are without antithyroid activity. Another idea proposed by the Mackenzies (personal communication) concerns the possibility that thiourea and related compounds may act by competing with iodide as a substrate for some oxidative enzyme system in the thyroid gland. It would be rash to conclude that all the thiourea and aminobenzene compounds act on the same mechanism in the thyroid gland. It is more likely that compounds, differing even slightly in chemical composition, operate in different ways to produce their antithyroid action.

3. *Effects on the Pituitary Thyrotropin Mechanism*

The level of the circulating thyroid hormone is one of the most important factors regulating the production and release of the pituitary thyrotropic hormone (Adams, 1946a). More particularly, a decrease in the concentration of circulating thyroxin, or the resulting depression of BMR, evokes an increased output of thyrotropin from the pituitary, which, in turn, causes hypertrophy and hyperplasia of the thyroid gland. This is the hypothesis which has been advanced for the goiters which result from the administration of the thiourea and aminobenzene compounds (the Mackenzies, 1943a; Astwood, Sullivan *et al.* 1943). This explanation is supported by the observation that thyrotropic hormone administration will duplicate all of the essential effects of thiouracil on the size and histology of the thyroid gland (Larson, Keating, Peacock, and Rawson, 1945a). The importance of the pituitary is seen also from the observation that hypophysectomy abolishes the thyroid response to the goitrogens, and from the histological alterations in the pituitary glands of the drug-treated animals (the Mackenzies, 1943a; Astwood, Sullivan, Bissell, and Tyslowitz, 1943; Gordon, Goldsmith, and Charipper, 1945b).

Actual assay of the hypophyses reveals a decreased thyrotropin content during administration of a rape seed diet (Griesbach and Purves, 1943), or following the feeding of thiourea or sulfadiazine (Gordon, Goldsmith, and Charipper, 1945a), a reaction due possibly to the augmented release of this hormone from the gland. There is difference of opinion, however, concerning the thyrotropin levels in the bloodstream in animals under goitrogenic stimulation. With the rape seed diet (Griesbach and Purves, 1943) an increased concentration has been reported, whereas with thiourea and sulfadiazine (Gordon, Goldsmith, and Charipper, 1945a) the blood level of this hormone is lowered. The latter finding has been interpreted in terms of increased removal and utilization of the released thyrotropin by the hyperplastic thyroid (Gordon, Goldsmith, and Charipper, 1945a), a phenomenon similar to the *in vitro* removal of thyrotropin by thyroid glands of normal and Graves' disease patients (Rawson, Sterne, and Aub, 1942; Rawson, Graham, and Riddell, 1943). Whether this difference in result is due to the type of treatment (*i.e.*, rape seed *vs.* thiourea or sulfadiazine), the length of treatment, or perhaps to the different kind of assay animal employed, should be determined.

Whatever the case may be, it seems certain that the thyroid hypertrophy and hyperplasia induced by the thioureas and other antithyroid substances are due largely to an increased production and release of thyrotropin from the anterior lobe of the hypophysis occurring as a compensatory measure following the inhibition of thyroid hormone synthesis. It is not likely that the changes induced in the pituitary are due to a direct action of the drugs on this gland, since cytological alterations in the anterior hypophysis are no different in thiourea-treated thyroidectomized than in untreated thyroidectomized rats (Griesbach and Purves, 1945). A direct enhancement, however, of the effect of thyrotropin by goitrogenic agents is seen in the recent interesting report by Rawson, Albert *et al.* (1946). As stated above, incubation of thyrotropic hormone with thyroid tissue results in inactivation of this principle. When the inactivated complex is treated with small amounts of thiouracil the active hormone is not only recovered but appears in greater concentration than in the original sample. On the basis of these results Rawson, Albert *et al.* have proposed (1946) that thiouracil exerts a double action: (1) inhibition of thyroid hormone synthesis, and (2) an augmentation of thyrotropic hormone activity.

4. Direct Effects on the Thyroid

A consideration of the mechanism of action of goitrogenic agents should take into account certain observations of hypophysectomized animals which indicate that these drugs may also directly affect the thyroid structure. For example, sulfaguanidine has been found to increase both the total protein and organic iodine concentration in the thyroidal colloid of hypophysectomized rats (Gersh and Baker, 1943). Further, Higgins and Ingle (1946) have observed effects on the thyroid colloid of hypophysectomized rats treated with promizole similar to those occurring in intact animals. In addition, the atrophic thyroids of tadpoles immersed in thiourea for long periods of time may respond abnormally to injections of thyrotropic hormone, indicating an eventual direct injury to the gland, at least in this species (Gordon, Goldsmith, and Charipper, 1945c).

VI. Effects on Organs Other than the Thyroid and Pituitary

1. Adrenal Gland

Kennedy and Purves (1941) reported hypertrophy of the adrenal cortex in rats fed *Brassica* seeds. This concurs with the observation that adrenotropic hormone action is augmented by thiouracil (Williams, Weinglass, Bissell, and Peters, 1944). Several of the more recent investigations, however, have indicated adrenal atrophy in animals treated with thioureas and sulfonamides (Leblond and Hoff, 1944; Leathem, 1945a). Depletion of cortical osmiophilic substance with thiourea has also been observed (Dalton, Morris, and Dubnik, 1945; Glock, 1945), and symptoms of adrenal cortical deficiency have been noted with thiourea and thiouracil (Glock, 1945). The oxygen consumption of adrenal tissue from rats fed thiouracil, however, is no different from that of normal adrenal tissue (Jandorf and Williams, 1944), nor are the androgenic or gluconeogenic activities of the adrenal in human patients altered by this drug (Williams, Bissell, Jandorf, and Peters, 1944). Sulfonamides (Endicott, Kornberg, and Daft, 1944), thiourea (Glock, 1945) or derivatives of thiouracil (McGavack and Vogel, 1945) eventually induce necrosis and hemorrhage in the adrenal cortex.

According to Marine and Baumann (1945a), hypertrophy of the adrenal medulla associated with a greater content of epinephrine occurs in rats treated chronically with thiouracil. In this connection an enhancement of the pressor action of adrenaline has been alleged to occur with isothiourea compounds (Fastier and Smirk, 1943). On the other hand, claims have been made that the thioureas antagonize the effect of adrenaline on the isolated uterus and intestine (Friedenwald and Buschke, 1943; Di Palma and Dreyer, 1945) and on the heart in the intact animal (Raab, 1944, 1945).

2. Reproductive Organs and Accessories

Antithyroid agents, in general, appear to exert a depressive influence on the reproductive system. For example, *Brassica* seed diets delay maturation of the ovary in immature rats (Kennedy and Purves, 1941). Similarly, decreases in size and weight have been observed for the ovaries, uteri (Dalton, Morris, and Dubnik, 1945), seminal vesicles and prostate glands (Leathem, 1945a) of rats fed thiourea, and an increase in length of the estrous cycle has been reported in rats under treatment with thiouracil (Mann, 1945). This drug also induces changes in the mammary gland similar to those occurring after thyroid removal (Smithcors, 1945). This is in accord with the observation that thiourea causes a reduction in milk output in the goat (Schultze and Turner, 1945).

Gametogenic function in both the male and female rat does not appear to be significantly affected by thiouracil treatment (Jones, Delfs, and Foote, 1946). In addition, thiourea given 1–15 days prior to parturition does not alter the gestation process in the rat (Goldsmith, Gordon, and Charipper, 1945b). Thiouracil, however, when fed both before mating and during pregnancy induces fetal resorption (Jones, Delfs, and Foote, 1946). An action on gametogenic function is generally reflected in a change in the gonadotropic hormone titer of the hypophysis. However, both an increase (Leathem, 1945a) and a decrease (Janes, 1946) in gonadotropic hormone potency of the rat pituitary have been reported following thiourea administration. Perhaps this discrepancy in result may be due to the differences in the concentration of the drug and lengths of treatment employed by these two workers. The action of chorionic gonadotrophin is not altered by thiouracil (Williams, Weinglass, Bissell, and Peters, 1944).

3. *Liver and Kidney*

The reports concerning the effects of antithyroid chemicals on the liver and kidney have not been uniform. As mentioned above, augmentation of liver weight and increased liver glycogen concentrations have been said to occur in rats under continuous treatment with sulfonamides or thiourea (Leblond and Hoff, 1944; Hueper and Ichniowski, 1944; May, Moseley, and Forbes, 1946). Increases in liver and kidney weights of chickens fed thiouracil have also been observed (Schultze and Turner, 1945). A marked decrease in liver glycogen concentration accompanied by a rise in the blood sugar level occurs, however, in rats given a single injection of α-naphthylthiourea (Du Bois, Holm, and Doyle, 1946). Depletion of liver glycogen has also been observed with thiourea (Glock, 1945). On the other hand, no significant alterations in liver and kidney weights or structure have been reported for thiouracil or thiourea (Williams, Weinglass, Bissell, and Peters, 1944; Leathem, 1945a). Similarly, there is no alteration in the oxygen consumption of liver tissue obtained from rats which had received thiouracil (Jandorf and Williams, 1944). The stimulatory effect of thyrotropic hormone on the oxygen uptake of liver, however, is abolished by simultaneous treatment with thiouracil (Jandorf and Williams, 1944). Recently, Tipton and Nixon (1946) have found a significant decrease in the activity of the succinoxidase and cytochrome oxidase systems in the livers of rats fed thiouracil. This action appears to be mediated through the thyroid since application of the drug directly to the tissue exerts no effect. The oxidase system of the kidney, however, is unaffected by thiourea or thiouracil (Paschkis, Cantarow, Rakoff, and Tillson, 1945b). Liver and kidney damage has been observed in rats given sulfonamides (Daft, Ashburn, and Sebrell, 1942; Ashburn, Daft, Endicott, and Sebrell, 1943; Endicott, Kornberg, and Daft, 1944) or thiouracil compounds (Meyer, Collins, and Marine, 1944; McGavack and Vogel, 1945; Vogel and McGavack, 1946). The livers and kidneys of the fowl appear to be resistant to even high dosages of thiouracil (Andrews and Schnetzler, 1946).

4. *Peripheral Blood and Bone Marrow*

Slight to moderate anemia has been a common finding in laboratory animals treated with sulfonamides (Daft, Ashburn, and Sebrell, 1942) or thiourea compounds (Hughes, 1944; Goldsmith, Gordon *et al.*, 1944; Williams, Weinglass *et al.*, 1944; Gordon, Goldsmith, and Charipper, 1945b; Higgins and Ingle, 1946; Vogel and McGavack, 1946; Daft, Kornberg *et al.*, 1946; Janes and Bradbury, 1946; Taber and Domm, 1946). Similarly, a delay in the regeneration of red cells following hemorrhage has been observed in rats fed thiouracil (Gordon, Kadow, Finkelstein, and

Charipper, 1946). The effects on the peripheral white cells are usually more drastic and generally include leucopenia or granulocytopenia or both (Daft, Ashburn, and Sebrell, 1942; Goldsmith, Gordon *et al.*, 1944; Williams, Weinglass *et al.*, 1944; Gordon, Goldsmith, and Charipper, 1945b; Daft, Kornberg *et al.*, 1946). The occasional occurrence of granulocytopenia in thyrotoxic patients under thiouracil therapy (Astwood, 1943a) is generally considered one of the most serious contraindications to its use. These blood dyscrasias disappear upon withdrawal of the drug and can be corrected or prevented by the administration of certain liver fractions (Kornberg, Daft, and Sebrell, 1943; Goldsmith, Gordon *et al.* 1944), folic acid (Daft and Sebrell, 1943; Daft, Kornberg *et al.*, 1946) or pyridoxine (Fishberg and Vorzimmer, 1945). It is still questionable, however, whether these agents have any value in the acute granulocytopenia induced by thiourea compounds in the hyperthyroid patient.

A decrease in the myeloid:erythroid cell ratio occurs in the bone marrow of rats treated with thiouracil (Vogel and McGavack, 1946). Sulfonamides, when administered as a single dose, do not affect the oxygen consumption of bone marrow (R̂er̂ábek, 1943). Likewise, Paschkis, Cantarow, Rakoff, and Tillson (1945b) have observed no influence on marrow oxidase with 0.002 M thiouracil, a concentration sufficient to depress the enzyme system in the thyroid. With larger concentrations of thiouracil, Warren (1945a, 1945b) has detected an inhibition of the *in vitro* respiration of the immature myeloid elements of the bone marrow; this effect could not be overcome by pyridoxine or liver extract. The bone marrow stores relatively large quantities of thiouracil (Williams, Kay, and Jandorf, 1944) and this may possibly be a factor in the susceptibility of this organ to such agents.

5. Other Organs

Damage to lung tissue has been observed with sulfonamides (Ashburn, Daft *et al.*, 1943) and thiourea compounds (Meyer, Collins, and Marine, 1944; McGavack and Vogel, 1945). Pulmonary edema, sometimes accompanied by pleural effusion, has also been reported with such drugs (Richter and Clisby, 1941; the Mackenzies, 1943b; Marine and Baumann, 1945b). The production of these effects is not dependent upon the presence of the thyroid (the Mackenzies, 1943b) or adrenal (Marine and Baumann, 1945b). However, thyroid feeding increases, and thyroidectomy decreases, the incidence of lung damage in thiourea-treated rats (Marine and Baumann, 1945b). Other organ changes include lesions in the pancreas, stomach, intestine, heart, blood vessels and muscle with the sulfonamides (Daft, Ashburn, and Sebrell, 1942; Ashburn, Daft *et al.*, 1943), hemosiderosis in the spleen with thiourea (Dalton, Morris, and Dubnik, 1946) and periarteritis nodosa with thiouracil (Marine and Baumann, 1945c). Thiourea apparently exerts no effect on autonomic nervous system activity in the intact animal (Di Palma and Dreyer, 1945). The increase in the Q_{O_2} of muscles caused by thyrotropin injections is overcome by thiouracil (Jandorf and Williams, 1944). This may possibly explain, to some extent, the greater fatigability of muscles in allythiourea-treated rats (Malcolm and Whitehead, 1944).

A complete interpretation is not available at the present time for this wide variety of effect on body organs induced by the antithyroid drugs. Little or no attempt has been made in many of the reports to distinguish those effects which result from the functional hypothyroidism induced by the drugs and those which represent simply a manifestation of the toxic activity of these compounds. Confusion is added by the numerous disparities in result reported by the different investigators.

VII. Factors Influencing Activity of Antithyroid Agents

1. Age

The young animal is more susceptible than the adult to the thyroid-enlarging effects of thiourea and related compounds (the Mackenzies, 1943a, 1943b; Astwood, 1944–5; Schultze and Turner, 1945). Several reports have also stressed the greater resistance of the immature animal to the toxic effects of the goitrogenic agents (the Mackenzies, 1943b; Dieke and Richter, 1945; McGavack and Vogel, 1945). Marine and Baumann (1945b), however, report the older rat better able to tolerate the toxic effects of thiourea. More detailed attempts should be made to relate these varied responses to the concentrations of the drug attained in the body fluids and tissues and to the levels of thyrotropin in the pituitary glands and blood sera in the different-aged animals.

2. Sex

No sex difference in response to goitrogenic agents has been observed in the rat (the Mackenzies, 1943a). In fowls, however, the goitrogenic action of thiourea compounds is more pronounced in the female than in the male (Schultze and Turner, 1945). In addition, the castrated male bird shows greater thyroid hypertrophy in response to thiouracil treatment than the normal male. These differences may be related to the sex hormones since estrogen can augment the goitrogenic and fattening effects of thiouracil in both the male and female fowl (Schultze and Turner, 1945; Schnetzler, Andrews, and Hauge, 1945). In the rat, however, neither estrogen nor androgen (Segaloff, 1944; Goldsmith, Gordon, and Charipper, 1945a) influences the goitrogenic activity of thiourea or thiouracil. Male rats are reported to withstand the toxic effects of thiourea more successfully than females (Marine and Baumann, 1945b).

3. *Diet*

As previously noted, cyanide compounds cannot induce their goitrogenic action when the iodine intake is high. Increased levels of dietary iodine, however, exert little effect on the activity of the thioureas and aminobenzene compounds. Astwood (1944–5), however, has demonstrated that a reduced iodine intake will potentiate the action of thiouracil in the rat. Thus a concentration of the drug which will scarcely cause thyroid enlargement when given in the normal diet, evokes a marked response when the dietary iodine level is lowered; this activity is abolished when the level of iodine is raised. Astwood (1944–5) has suggested that the difference between the cyanogen and thiourea or aminobenzene compounds may not be as sharp as suspected but merely one of degree, with the former substances acting as weaker goitrogens and requiring a medium of low iodine concentration for their activity. It is interesting to speculate that perhaps differences in the response to iodine prophylaxis may be explained in terms of the amounts of possible endogenous goitrogen present at the time within the organism (Hellwig, 1935; McCarrison, 1937).

The Mackenzies (1943b) were the first to suggest that dietary factors may be important in the resistance of animals to the toxic effects of thiourea and related compounds. This has been confirmed (Dieke and Richter, 1945; Landgrebe and Morgan, 1946) and strengthened by the observation that iodine (Griesbach, Kennedy, and Purves, 1944; Byerrum, 1946) and cysteine (DuBois, Holm, and Doyle, 1946) will provide protection to rats against thiourea compounds. Similarly, a low casein diet will accentuate the toxic effects of sulfadiazine on rat kidney (Kornberg, Endicott *et al.*, 1945) and addition of sulfonamides to purified diets will induce abnormalities traceable to distinct vitamin deficiencies (Daft, Ashburn, and Sebrell, 1942; Daft, Endicott *et al.*, 1943; Kornberg, Daft, and Sebrell, 1944).

4. *Temperature*

Low temperatures augment, while higher ones diminish, the thyroid response to thiouracil (Dempsey and Astwood, 1943; Paschkis, Cantarow, Rakoff, and Rothenberg, 1945). This is most likely a manifestation of the different needs of the organism for thyroid hormone at the different temperatures (Leblond and Gross, 1943).

5. *Method of Administration*

The goitrogenic agents have proved effective whether given orally, parenterally or even topically (Williamson, Warren, and Werner, 1945).

Most of the thiourea compounds are more active when administered orally than when injected (Astwood, 1944–5). Mercaptothiazoline, however, is an antithyroid drug which appears to be more active subcutaneously than orally (Astwood, 1944–5). Frequent administration of these agents gives more pronounced effects than single large doses (Williams, 1944a; Paschkis, Cantarow, Rakoff, and Tillson, 1945a). Preparation and implantation of pellets of antithyroid chemicals should be attempted to determine whether slower absorption might yield more pronounced effects. It is important to know whether or not differences in rate of absorption, excretion, or breakdown of the essential chemical grouping, may be factors which determine the relative effectiveness of the various antithyroid drugs.

6. *Species Differences*

Differences exist in the resistance displayed by different species to thiourea compounds. Thus, the rabbit has been reported as less resistant than the rat to S-methylisothiourea (Hueper and Ichniowski, 1944). The lethal dose of α-naphthylthiourea in the rabbit, mouse, ground squirrel and prairie dog is 10–100 times that in the Norway rat (Phillips, 1946). Enormous differences exist even among animals of the same species. The wild Norway rat, for example, can tolerate as much as 300 times the lethal dose for tame Norways (Dieke and Richter, 1945). Diet probably plays a role in the resistance of the different species to the different drugs (the Mackenzies, 1943b; Dieke and Richter, 1945; Landgrebe and Morgan, 1946).

VIII. METABOLISM OF ANTITHYROID AGENTS

With the aid of chemical tests for the identification of thiourea compounds in body fluids and tissues (Campbell, Landgrebe, and Morgan, 1944; Danowski, 1944; Chesley, 1944a; Williams, Jandorf, and Kay, 1944), considerable light has been shed on the fate of these agents within the organism. The experiments of Williams and his collaborators (Williams, Jandorf, and Kay, 1944; Williams and Kay, 1945) and others (Campbell, Landgrebe, and Morgan, 1944; Paschkis, Cantarow, Rakoff, and Tillson, 1945a) have indicated that thiouracil and thiourea are rapidly absorbed from the gastrointestinal tract following oral administration. The most active sites of absorption are the stomach and duodenum and only about 15% of the ingested drug is destroyed in the alimentary tract (Williams and Kay, 1944; Williams, Kay, and Jandorf, 1944). In patients on routine thiouracil treatment, a marked increase in the concentration of the drug in the blood occurs from 1 to 2 hours following administration (Williams, 1944a). In the intervals between drug feeding, the concentration decreases rapidly. Essentially the same results follow thiouracil administration to the fowl (Pipes and Turner, 1946).

The quantity of drug excreted varies with the size and number of doses; when given several times daily, about one-third is excreted in the urine (Williams and Kay, 1944) and none appears in the stool (Williams and Kay, 1944; Williams, 1944b; Chesley, 1944b). The remainder of the drug is rapidly distributed throughout the tissues and fluids of the body (Williams and Kay, 1944; Campbell, Landgrebe, and Morgan, 1944). Largest concentrations of the drug are detected in bone marrow, thyroid, ovary, pituitary and adrenal; only small quantities appear in the muscles and liver. The presence of relatively large amounts of thiouracil in the thyroid gland has considerable significance and may partly explain its marked effects on the activity of this organ. In this connection, it has been found (Williams, Weinglass, and Kay, 1944) that thyrotropic hormone decreases the thiouracil content of the thyroid and KI increases its storage. The suggestion has been made (Williams, Weinglass, and Kay, 1944) that iodine may potentiate the action of thiouracil by facilitating its storage in the thyroid gland. Iodine administration, however, does not reduce the thiouracil requirement in thyrotoxic patients (Williams and Clute, 1945).

Following its administration, thiouracil can be found not only in the blood but also in the milk, where it usually appears in greater amounts, and in the pleural, pericardial, ascitic and cerebrospinal fluids (Williams, Kay, and Jandorf, 1944; Chesley, 1944b). In general, the body fluids of mammals contain less of the administered drug than do the tissues. In fowl, however, thiouracil does not concentrate to any greater extent in the tissues than in the blood (Pipes and Turner, 1946). This may account for the small amount of tissue damage in birds fed dosages of the drug injurious to the mammal (Andrews and Schnetzler, 1946). In blood, thiouracil appears bound to protein and more is present in the blood cells than in the plasma (Williams and Kay, 1944; Williams, Kay, and Jandorf, 1944). It may be recovered from serum by dialysis (Paschkis, Cantarow, Rakoff, and Tillson, 1945a). No direct connection between the protein-binding capacity of several antithyroid agents and their ability to inhibit thyroid function has been observed (Christensen, 1946).

Incubation of thiouracil with various body tissues results in its inactivation. It is interesting to note that on a unit weight basis pituitary destroys more of the drug than any other tissue; thyroid is second in effectiveness and adrenal third. Liver, kidney, pancreas and muscle exert little effect on the activity of thiouracil (Williams and Kay, 1944; Williams, Kay, and Jandorf, 1944).

Although an increased excretion of neutral sulphur and a decrease in the excretion of inorganic and etheral sulphur follow administration of thiouracil to normal people, the precise nature of the decomposition products of such drugs has not as yet been identified (Williams and Kay, 1944).

IX. CHEMICAL NATURE OF ANTITHYROID AGENTS

Space does not permit a detailed discussion of the extensive work on the chemical structure and relationships of the several hundred compounds which have been tested recently for antithyroid activity, and the reader is referred to some key references on this subject (the Mackenzies, 1943a; Astwood, Sullivan *et al.*, 1943; Astwood, 1943b; Schachner, Franklin, and Chaikoff, 1943; Astwood, 1944–1945; Taurog, Chaikoff, and Franklin, 1945; Astwood, Bissell, and Hughes, 1945; McGinty and Bywater, 1945a; Bywater, McGinty, and Jenesel, 1945; McGinty and Bywater, 1945b; Williams and Frame, 1945; Anderson, Halverstadt *et al.*, 1945; Chen, Harris, and Anderson, 1946; VanderLaan and Bissell, 1946a). In general, the active substances fall into one of three classes: (1) thiourea and its derivatives, (2) aniline derivatives, including the sulfonamides and other aminobenzene compounds, (3) thiocyanates and organic cyanides. Most of the recent experiments have centered about the first 2 groups of compounds, mainly because of their greater activity and because the third group is more toxic, and effective only in the presence of low levels of iodine. The methods employed for the determination of the activity of the antithyroid agents have generally consisted in administering the compound in the food or water of rats or chicks for 10–14 days and then sacrificing the animals for ascertainment of: (1) degree of thyroid enlargement, and (2) extent of thyroid hyperplasia and colloid loss, or (3) extent of iodine depletion within the thyroid gland. A fourth method, based on the ability of the various drugs to inhibit the *in vitro* incorporation of radioactive iodide into diiodotyrosine and thyroxine by thyroid tissue, has also been employed (Taurog, Chaikoff, and Franklin, 1945), but such a technique does not necessarily yield information as to the activity of the compound within the organism.

A short summary of the more pertinent facts concerning the chemical nature of the recently identified compounds may not be amiss. The thioureylene compounds, as a group, are more potent antithyroid agents than the aniline or aminobenzene derivatives. Replacement of the hydrogens of thiourea by methyl groups increases its activity approximately 300%, which suggests the importance of the thio rather than the mercapto grouping for the activity of this class of substances. The importance of S in the compound is shown by the fact that activity disappears when the thio group is replaced by other radicals as in urea or guanidine. Both amino nitrogens are not essential, since certain five-membered heterocyclic ring compounds, such as 2-mercaptothiazoline and 2-mercaptooxazoline which have S and O groups, respectively, replacing one of the amino nitrogens, possess even greater activity than thiourea. Substitution of aromatic or aliphatic radicals for one or more hydrogens may decrease or even abolish its activity. Incorporation of thiourea into a six membered ring may significantly increase its antithyroid effect as in thiouracil (2-thio-4-oxypyrimidine), which is about 10 times more active than thiourea.

The most potent antithyroid compounds are found among the derivatives of thiouracil. Thus, replacement of the hydrogen at the 5th or 6th position in thiouracil with certain hydrocarbon groups may increase strikingly its effect, as with 6-*n*-propyl and 6-ethylthiouracil which are 11 and 8 times more potent, respectively, than thiouracil. The 2-thiobarbituric acids are active when the hydrogens at position 5 are replaced by alkyl groups, the most effective thus far tested being the 5,5′-diethyl derivative (thiobarbital). The general anesthetic property possessed by these compounds is not responsible for their activity since other powerful hypnotic 2-thiobarbituric acid derivatives lack antithyroid capacity.

The thiocarbamyl grouping appears necessary for the activity of the aniline or aminobenzene derivatives. Among the compounds in this series more potent than sulfadiazine are 4,4′-diaminodiphenylmethane, its tetramethyl derivative and promizole (2-amino-5-sulfanilylthiazole). These compounds, however, are only about $\frac{1}{4}$ to $\frac{1}{2}$ as active as thiouracil; 4-aminobenzoic acid possesses only about $\frac{1}{30}$th the potency of thiouracil.

It should be borne in mind that the majority of the results on the relative activity of the various antithyroid compounds have been based on their assay in the rat. It is conceivable that a different order of effectiveness might obtain were another type of assay animal to be employed.[3] Thus, for example, thiouracil is considerably more active than thiourea in the rat and man. But in the chick (VanderLaan and Bissell, 1946a), the activity of thiourea, at certain dosage levels, is equal to that of thiouracil, and in ruminants and certain domestic fowls (Schultze and Turner, 1945) thiourea is actually more goitrogenic than thiouracil. As Astwood (1944–5) and Williams and Frame (1945) have emphasized, caution must be taken in applying the results obtained in one type of animal to another, such as man, for here the effectiveness, toxicity, and fate of the compound may be of an entirely different order. Apart from the activity of its chemical grouping *per se* the effectiveness of a compound may depend on such variables as its solubility, toxicity, mode and frequency of administration, quantity of iodine in the diet, the environmental temperature, humidity and other seasonal factors as well as the criteria used for measuring its potency. Moreover, an animal could refrain from consuming normal amounts of a diet containing a drug with a pronounced disagreeable taste or odor. Factors such as these may possibly account for the somewhat different order of activity found for certain of the antithyroid drugs by different investigators.

---

[3] See recent paper by Stanley, M. M., aud Astwood, E. B. 1947. *Endocrinology* **41**, 66–84.

The report by Carter, Mann, Harley-Mason, and Jenkins (1943) that *p*-xanthine (1,7-dimethylxanthine) is a powerful naturally-occurring (Carter and Jenkins, 1944) antithyroid substance, which acts by antagonizing thyroxine directly, has aroused considerable interest. Recent work (Gordon, Charipper, and Goldsmith, 1944; Williams and Peters, 1945; Barker, 1945b; Paschkis, Cantarow, and Tillson, 1945), however, has not confirmed this finding and has shown, in addition, that this compound exerts no effect upon the metamorphosis of amphibia, the metabolic rate of rats and their resistance to reduced pressures, the cytochrome oxidase system of the thyroid gland, or the condition of a thyrotoxic patient.

X. Clinical Applications

The recent intensive efforts which have been made in the preparation and testing of many antithyroid compounds have been primarily for the purpose of discovering agents which can be employed for the effective treatment of hyperthyroidism in man. The striking success obtained clinically with certain thiourea compounds, particularly thiouracil, has more than repaid these efforts and has shown convincingly once again the profits to be reaped from a close cooperation, in problems of this type, between the organic chemist, pharmacologist and clinician. Since the initial report by Astwood (1943a) describing the beneficial results obtained with thiourea and thiouracil in the treatment of typical Graves' disease, many laboratories and clinics in this country and several in Europe and South America have also reported favorable results with such agents. Several illuminating papers (Astwood, 1943a; Williams and Bissell, 1943; Rawson, Evans *et al.*, 1944; Williams, Bissell *et al.* 1944; Williams and Clute, 1944; Astwood, 1944; McGavack, Gerl *et al.*, 1944; McGavack, Gerl *et al.* 1945; Thompson and Thompson, 1945; Jackson, 1945; Bartels, 1945; Van Winkle, Hardy *et al.*, 1946; Williams, Clute *et al.*, 1946) should be consulted for details on this subject.

Thiouracil has proved more satisfactory in humans than thiourea and has been the drug most extensively used thus far. Recently, however, 6-ethylthiouracil, 6-*n*-propylthiouracil (Astwood and VanderLaan, 1945) and 6-*n*-isobutylthiouracil (Williams, 1946) have been found to be more potent and probably less toxic than thiouracil in thyrotoxic patients. The thiouracil compounds are markedly effective in primary hyperthyroidism, noticeable improvement occurring, with proper dosage, within one week. By the end of 4–6 weeks of treatment, practically all signs of thyrotoxicosis have disappeared. This includes amelioration of such symptoms as the tachycardia, palpitation, tremor, dyspnea and nervousness, disappearance of the metabolic and biochemical derangements associated with the disease, reduction of the protein-bound iodine in the plasma, and improvement of the psychic and mental outlook of the patient. Exophthalmos of the nonmalignant type generally improves as the thyrotoxic symptoms wane. After prolonged treatment with thiouracil, remissions may occur, lasting, in some cases, for as long as several months after discontinuation of the drug. This is especially important since it indicates that these agents may eventually result in a permanent return of the faulty pituitary-thyroid mechanism to a more normal state.

Conflicting reports have appeared on the behavior of the goiter in patients under thiouracil treatment. Most agree, however, that with prolonged therapy it tends eventually to regress. Thiouracil, in many cases, has proved even more effective than iodine in the preparation of patients for thyroid removal. In cases of toxic adenoma, which generally requires a 2-stage operation, only one stage is necessary now, provided thiouracil is employed as a preoperative measure. Technical difficulties may arise in the use of thiouracil for this purpose, since the drug has been reported to cause increased vascularity and friability of the gland, but when it is administered in conjunction with iodide, this difficulty is largely overcome.

A good deal has been written about the toxic reactions which attend the administration of thiourea and related compounds to thyrotoxic patients, but these have occurred in only 10% of the cases and less than 1% have succumbed, a mortality figure less than that resulting from surgical removal of the gland in Graves' disease. The untoward reactions generally appear within the first month and include fever, skin rash, arthritis, diarrhea, jaundice, edema, gastrointestinal disturbances, enlargement of salivary glands, lymphadenopathy, leucopenia and granulocytopenia. Usually only the last condition has proven sufficiently serious to warrant additional palliative measures or discontinuation of treatment. Several of the other toxic symptoms may be due simply to idiosyncratic reactions developed by the patient to the thiouracil-protein complexes formed within the body (McArthur, Rawson, and Means, 1945).

In view of the recent findings (Bielschowsky, 1944; 1945; Griesbach, Kennedy, and Purves, 1945; Dalton, Morris, and Dubnik, 1946) that thyroid neoplasms may arise in animals under treatment for long periods of time with rape seed and thiourea compounds, attention should be paid to the possible occurrence of such abnormal growths in hyperthyroid patients treated chronically with antithyroid drugs.

XI. CONCLUSION

The recent work on the antithyroid agents has provided a new approach to the study of thyroid physiology. It has not only made available effective chemical weapons for combating toxic goiter but has also made material contribution to a further understanding of the basic reactions underlying the functioning of the thyroid gland and its relation to the hypophyseal-thyrotrophic mechanism. The new drugs have also proved of value as a means of determining the rate of thyroxine secretion by the thyroid glands of rats and birds under varying environmental conditions and under diverse physiological states of activity (Dempsey and Astwood, 1943; Schultze and Turner, 1944, 1945; Reineke, Mixner, and Turner, 1945; Reineke and Turner, 1945; Higgins and Joneson, 1946). Further, they have afforded a means of increasing the tolerance of animals to anoxia or lowered barometric pressure (Lawson, 1942; Gordon, Goldsmith, and Charipper, 1944; Leblond, 1944; Hughes and Astwood, 1944b; Goldsmith, Gordon, and Charipper, 1945a; Gordon, Goldsmith, and Charipper, 1945b). They are considered of value in the treatment of angina pectoris (Raab, 1944, 1945) and in the prevention of experimental dietary cirrhosis of the liver (György and Goldblatt, 1945). The marrow-depressing effect of thiouracil has suggested its use in leukemia (Limarzi, Pirani, and Kulsavage, 1945). Advantage has been taken of the highly toxic nature of several derivatives of thiourea, particularly α-naphthylthiourea (Richter, 1945; Phillips, 1946), in their employment as potent rodenticides.

Many problems relating to thyroid activity, under normal and abnormal conditions, still remain to be solved. It is most certain, however, that with the interest still mounting in the search for new antithyroid compounds and their mechanism of action, the continued employment of such agents as an experimental tool should prove even more rewarding in the future than it has in the past.

REFERENCES

1. Adams, A. E. 1946a. *Quart. Rev. Biol.* **21,** 1–32.
2. Adams, A. E. 1946b. *Anat. Record, Suppl.* **94,** 88.
3. Allen, B. M. 1918. *J. Exptl. Zoöl.* **24,** 499–520.
4. Anderson, G. W., Halverstadt, I. F., Miller, W. H., and Roblin, R. O., Jr. 1945. *J. Am. Chem. Soc.* **67,** 2197–2200.
5. Andrews, F. N., and Schnetzler, E. E. 1945. *Endocrinology* **37,** 382–4.
6. Andrews, F. N., and Schnetzler, E. E. 1946. *Poultry Sci.* **25,** 124–9.
7. Aranow, H., Jr., Engle, E. T., and Sperry, W. M. 1946. *Endocrinology* **38,** 331–6.
8. Ashburn, L. L., Daft, F. S., Endicott, K. M., and Sebrell, W. H. 1943. *U. S. Pub. Health Service, Publ. Health Rept.* **57,** 1883–91.
9. Astwood, E. B. 1943a. *J. Am. Med. Assoc.* **122,** 78–81.
10. Astwood, E. B. 1943b. *J. Pharmacol.* **78,** 79–89.

11. Astwood, E. B. 1944. *J. Clin. Endocrinol.* **4,** 229–48.
12. Astwood, E. B. 1944–45. Chemotherapy of Hyperthyroidism. Harvey Lectures, pp. 195–235.
13. Astwood, E. B., and Bissell, A. 1944. *Endocrinology* **34,** 282–96.
14. Astwood, E. B., Bissell, A., and Hughes, A. M. 1944. *Federation Proc.* **3,** 2.
15. Astwood, E. B., Bissell, A., and Hughes, A. M. 1945. *Endocrinology* **37,** 456–81.
16. Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R. 1943. *Endocrinology* **32,** 210–25.
17. Astwood, E. B., and VanderLaan, W. P. 1945. *J. Clin. Endocrinol.* **5,** 424–30.
18. Barker, M. H., Lindberg, H. A., and Wald, M. H. 1941. *J. Am. Med. Assoc.* **117,** 1591–4.
19. Barker, S. B. 1945a. *Endrocrinology* **37,** 230–6.
20. Barker, S. B. 1945b. *Endocrinology* **37,** 484–6.
21. Barr, D. P., and Shorr, E. 1945. *Ann. Internal Med.* **23,** 754–78.
22. Bartels, E. C. 1945. *Ann. Internal Med.* **22,** 365–72.
23. Baumann, E. J., Metzger, N., and Marine, D. 1944. *Endocrinology* **34,** 44–9.
24. Bevelander, G. 1946. *Proc. Soc. Exptl. Biol. Med.* **61,** 268–70.
25. Bielschowsky, F. 1944. *Brit. J. Exptl. Path.* **25,** 90–5.
26. Bielschowsky, F. 1945. *Brit. J. Exptl. Path.* **26,** 270–5.
27. Borland, J. R. 1943. *J. Exptl. Zoöl.* **94,** 115–44.
28. Briggs, G. M., Jr., and Lillie, R. J. 1946. *Proc. Soc. Exptl. Biol. Med.* **61,** 430–2.
29. Byerrum, R. V. 1946. *Proc. Soc. Exptl. Biol. Med.* **62,** 328–30.
30. Bywater, W. G., McGinty, D. A., and Jenesel, D. A. 1945. *J. Pharmacol.* **85,** 14–22.
31. Campbell, D., Landgrebe, F. W., and Morgan, T. N. 1944. *Lancet* **246,** 630–2.
32. Carter, G. S., and Jenkins, G. N. 1944. *Nature* **154,** 639.
33. Carter, G. S., Mann, F. G., Harley-Mason, J., and Jenkins, G. N. 1943. *Nature* **151,** 728–30.
34. Chagas, C., De Robertis, E., and Couceiro, A. 1945. *Texas Rept. Biol. Med.* **3,** 170–8.
35. Chapman, A. 1941. *Endocrinology* **29,** 686–94.
36. Chen, K. K., Harris, P. N., and Anderson, R. C. 1946. *Federation Proc.* **5,** 170–1.
37. Chesley, L. C. 1944a. *J. Biol. Chem.* **152,** 571–8.
38. Chesley, L. C. 1944b. *J. Clin. Investigation* **23,** 856–8.
39. Chesney, A. M., Clawson, T. A., and Webster, B. 1928. *Bull. Johns Hopkins Hosp.* **43,** 261–77.
40. Christensen, B. G. 1945. *Acta Pharmacol. Toxicol.* **1,** 98–105.
41. Christensen, H. N. 1946. *J. Biol. Chem.* **162,** 27–35.
42. Couceiro, A., and Vieira, L. G. 1944. *Rev. brasil. biol.* **4,** 323–7.
43. Couceiro, A., Vieira, L. G., and de Moraes, J. 1944. *Rev. brasil. biol.* **4,** 173–84.
44. Daft, F. S., Ashburn, L. L., and Sebrell, W. H. 1942. *Science* **96,** 321–2.
45. Daft, F. S., Endicott, K. M., Ashburn, L. L., and Sebrell, W. H. 1943. *Proc. Soc. Exptl. Biol. Med.* **53,** 130–1.
46. Daft, F. S., Kornberg, A., Ashburn, L. L., and Sebrell, W. H. 1946. *Proc. Soc. Exptl. Biol. Med.* **61,** 154–8.
47. Daft, F. S., and Sebrell, W. H. 1943. *U. S. Pub. Health Service, Pub. Health Rept.* **58,** 1542–5.
48. Dalton, A. J., Morris, H. P., and Dubnik, C. S. 1945. *J. Natl. Cancer Inst.* **5,** 451–4.

49. Dalton, A. J., Morris, H. P., and Dubnik, C. S. 1946. *Federation Proc.* **5,** 219.
50. Danowski, T. S. 1944. *J. Biol. Chem.* **152,** 201–5.
51. Danowski, T. S., Man, E. B., and Winkler, A. W. 1946. *Endocrinology* **38,** 230–7.
52. Dempsey, E. W. 1944. *Endocrinology* **34,** 27–38.
53. Dempsey, E. W., and Astwood, E. B. 1943. *Endocrinology* **32,** 509–18.
54. Dempsey, E. W., and Singer, M. 1946. *Endocrinology* **38,** 270–95.
55. De Robertis, E. 1941. *Anat. Record* **80,** 219–31.
56. De Robertis, E., and Gonçalves, J. M. 1945. *Endocrinology* **36,** 245–50.
57. De Robertis, E., and Grasso, R. 1946. *Endocrinology* **38,** 137–46.
58. De Robertis, E., and Nowinski, W. W. 1945. *Rev. soc. argent. biol.* **21,** 120–35.
59. Dieke, S. H., and Richter, C. P. 1945. *J. Pharmacol.* **83,** 195–202.
60. Dietrich, W. C., and Beutner, R. 1944. *Proc. Soc. Exptl. Biol. Med.* **57,** 35–36.
61. Di Palma, J. R., and Dreyer, N. B. 1945. *Endocrinology* **36,** 236–8.
62. Domm, L. V., and Blivaiss, B. B. 1944. *Proc. Soc. Exptl. Biol. Med.* **57,** 367–8.
63. Domm, L. V., and Blivaiss, B. B. 1946. *Anat. Record, Suppl.* **94,** 68–9.
64. Drill, V. A. 1943. *Physiol. Revs.* **23,** 335–79.
65. Du Bois, K. P., Holm, L. W., and Doyle, W. L. 1946. *Proc. Soc. Exptl. Biol. Med.* **61,** 102–4.
66. Dziemian, A. 1943. *J. Cellular Comp. Physiol.* **21,** 339–45.
67. Endicott, K. M., Kornberg, A., and Daft, F. S. 1944. *U. S. Pub. Health Service, Pub. Health Rept.* **59,** 49–54.
68. Engle, E. T., and Aranow, H., Jr. 1946. *Endocrinology* **38,** 325–30.
69. Fastier, F. N., and Smirk, F. H. 1943. *J. Physiol.* **101,** 379–88.
70. Fishberg, E. H., and Vorzimmer, J. 1945. *Proc. Soc. Exptl. Biol. Med.* **60,** 181–4.
71. Foulger, M. P. H., and Rose, E. 1943. *J. Am. Med. Assoc.* **122,** 1072–3.
72. Franklin, A. L., and Chaikoff, I. L. 1943. *J. Biol. Chem.* **148,** 719–20.
73. Franklin, A. L., and Chaikoff, I. L. 1944. *J. Biol. Chem.* **152,** 295–301.
74. Franklin, A. L., Chaikoff, I. L., and Lerner, S. R. 1944. *J. Biol. Chem.* **153,** 151–62.
75. Friedenwald, J. S., and Buschke, W. 1943. *Am. J. Physiol.* **140,** 360–73.
76. Gersh, I., and Baker, R. F. 1943. *J. Cellular Comp. Physiol.* **21,** 213–27.
77. Glock, G. E. 1944. *Nature* **154,** 460–61.
78. Glock, G. E. 1945. *Nature* **156,** 508.
79. Goldsmith, E. D., Gordon, A. S., and Charipper, H. A. 1944. *Federation Proc.* **3,** 13.
80. Goldsmith, E. D., Gordon, A. S., and Charipper, H. A. 1945a. *Endocrinology* **36,** 364–9.
81. Goldsmith, E. D., Gordon, A. S., and Charipper, H. A. 1945b. *Am. J. Obstet. Gynecol.* **49,** 197–206.
82. Goldsmith, E. D., Gordon, A. S., Finkelstein, G., and Charipper, H. A. 1944. *J. Am. Med. Assoc.* **125,** 847.
83. Goldsmith, E. D., and Harnly, M. H. 1946. *Science* **103,** 649–51.
84. Goldsmith, E. D., Nigrelli, R. F., Gordon, A. S., Charipper, H. A., and Gordon, M. 1944. *Endocrinology* **35,** 132–4.
85. Gordon, A. S., Charipper, H. A., and Goldsmith, E. D. 1944. *Anat. Record Suppl.* **89,** 38.
86. Gordon, A. S., Goldsmith, E. D., and Charipper, H. A. 1943. *Nature* **152,** 504–5.

87. Gordon, A. S., Goldsmith, E. D., and Charipper, H. A. 1944. *Science* **99,** 104–5.
88. Gordon, A. S., Goldsmith, E. D., and Charipper, H. A. 1945a. *Endocrinology* **36,** 53–61.
89. Gordon, A. S., Goldsmith, E. D., and Charipper, H. A. 1945b. *Endocrinology* **37,** 223–9.
90. Gordon, A. S., Goldsmith, E. D., and Charipper, H. A. 1945c. *Growth* **9,** 19–41.
91. Gordon, A. S., Goldsmith, E. D., and Charipper, H. A. 1946. *Am. J. Physiol.* **146,** 439–42.
92. Gordon, A. S., Kadow, P. C., Finkelstein, G., and Charipper, H. A. 1946. *Am. J. Med. Sci.* **212,** 385–394.
93. Griesbach, W. E. 1941. *Brit. J. Exptl. Path.* **22,** 245–9.
94. Griesbach, W. E., Kennedy, T. H., and Purves, H. D. 1941. *Brit. J. Exptl. Path.* **22,** 249–54.
95. Griesbach, W. E., Kennedy, T. H., and Purves, H. D. 1944. *Nature* **154,** 610–11.
96. Griesbach, W. E., Kennedy, T. H., and Purves, H. D. 1945. *Brit. J. Exptl. Path.* **26,** 18–24.
97. Griesbach, W. E., and Purves, H. D. 1943. *Brit. J. Exptl. Path.* **24,** 174–84.
98. Griesbach, W. E., and Purves, H. D. 1945. *Brit. J. Exptl. Path.* **26,** 13–7.
99. György, P., and Goldblatt, R. 1945. *Science* **102,** 451–2.
100. György, P., Stiller, E. T., and Williamson, M. B. 1943. *Science* **98,** 518–20.
101. Harington, C. R. 1944. *Proc. Roy. Soc. London* **B132,** 223–8.
102. Hartzell, A. 1945. *Contrib. Boyce Thompson Inst.* **13,** 501–13.
103. Hellwig, C. A. 1935. *Arch. Path.* **19,** 364–71.
104. Hercus, C. E., and Purves, H D. 1936. *J. Hyg.* **36,** 182–203.
105. Higgins, G. M. 1945. *Am. J. Med. Sci.* **210,** 347–62.
106. Higgins, G. M., and Ingle, D. J. 1946. *Endocrinology* **38,** 110–21.
107. Higgins, G. M., and Joneson, O. R. 1946. *Anat. Record Suppl.* **94,** 24.
108. Hoskins, E. R., and Hoskins, M. M. 1919. *J. Exptl. Zoöl.* **29,** 1–69.
108a. Houssay, B. A. 1946. *Vitamins & Hormones* **4,** 188–206.
109. Hueper, W. C., and Ichniowski, C. T. 1944. *Arch. Path.* **37,** 253–63.
110. Hughes, A. M. 1944. *Endocrinology* **34,** 69–74.
111. Hughes, A. M., and Astwood, E. B. 1944a. *Endocrinology* **34,** 138–9.
112. Hughes, A. M., and Astwood, E. B. 1944b. *J. Aviation Med.* **15,** 152–7.
113. Jackson, A. S. 1945. *Am. J. Surg.* **67,** 467–78.
114. Jailer, J. W., Sperry, W. M., Engle, E. T., and Smelser, G. 1944. *Endocrinology* **35,** 27–37.
115. Jandorf, B. J., and Williams, R. H. 1944. *Am. J. Physiol.* **141,** 91–6.
116. Janes, R. G. 1946. *Anat. Record Suppl.* **94,** 29.
117. Janes, R. G., and Bradbury, J. T. 1946. *Anat. Record Suppl.* **94,** 68.
118. Johnson, T. B., and Tewkesbury, L. B. 1942. *Proc. Natl. Acad. Sci. U. S.* **28,** 72–7.
119. Jones, G. E. S., Delfs, E., and Foote, E. C. 1946. *Endocrinology* **38,** 337–44.
120. Juhn, M. 1944. *Endocrinology* **35,** 278–9.
121. Juhn, M. 1946. *Endocrinology* **39,** 14–22.
122. Keating, F. R., Jr., Rawson, R. W., Peacock, W., and Evans, R. D. 1945. *Endocrinology* **36,** 137–48.
123. Kempster, H. L., and Turner, C. W. 1945. *Poultry Sci.* **24,** 94–6.

124. Kennedy, T. H. 1942. *Nature* **150,** 233–4.
125. Kennedy, T. H., and Purves, H. D. 1941. *Brit. J. Exptl. Path.* **22,** 241–4.
126. Keston, A. S. 1944. *J. Biol. Chem.* **153,** 335–6.
127. Keston, A. S., Goldsmith, E. D., Gordon, A. S., and Charipper, H. A. 1944. *J. Biol. Chem.* **152,** 241–4.
128. Kornberg, A., Daft, F. S., and Sebrell, W. H. 1943. *Science* **98,** 20–2.
129. Kornberg, A., Daft, F. S., and Sebrell, W. H. 1944. *U. S. Pub. Health Service, Pub. Health Rept.* **59,** 832–44.
130. Kornberg, A., Endicott, K. M., Daft, F. S., and Sebrell, W. H. 1945. *U. S. Pub. Health Service, Pub. Health Rept.* **60,** 661–75.
131. Landgrebe, F. W., and Morgan, T. N. 1946. *Nature* **157,** 22–3.
132. Larson, R. A., Keating, F. R., Jr., Peacock, W., and Rawson, R. W. 1945a. *Endocrinology* **36,** 149–59.
133. Larson, R. A., Keating, F. R., Jr., Peacock, W., and Rawson, R. W. 1945b. *Endocrinology* **36,** 160–9.
134. Lawson, F. L. 1942. *Am. J. Physiol.* **136,** 494–505.
135. Leathem, J. H. 1945a. *Endocrinology* **36,** 98–103.
136. Leathem, J. H. 1945b. *Endocrinology* **37,** 482–3.
137. Leathem, J. H. 1946. *Federation Proc.* **5,** 61.
138. Leblond, C. P. 1944. *Proc. Soc. Exptl. Biol. Med.* **55,** 114–6.
139. Leblond, C. P., and Gross, J. 1943. *Endrocrinology* **33,** 155–60.
140. Leblond, C. P., and Hoff, H. E. 1944. *Endocrinology* **35,** 229–33.
141. Lerner, S. R., and Chaikoff, I. L. 1945. *Endocrinology* **37,** 362–8.
142. Limarzi, L. R., Pirani, C. L., and Kulsavage, R. J. 1945. *Proc. Cent. Soc. Clin. Res.* **18,** 18–9.
142a. Lowenstein, B. E., Bruger, M., Hinton, J. W., and Lough, W. G. 1945. *J. Clin. Endocrinol.* **5,** 181–3.
143. Lynn, G. W., and De Marie, A. 1946. *Science* **104,** 31.
144. Mackenzie, C. G., and Mackenzie, J. B. 1943a. *Endocrinology* **32,** 185–209.
145. Mackenzie, J. B., and Mackenzie, C. G. 1943b. *Proc. Soc. Exptl. Biol. Med.* **54,** 34–7.
146. Mackenzie, J. B., and Mackenzie, C. G. 1942. *Federation Proc.* **1,** 122–3.
147. Mackenzie, J. B., and Mackenzie, C. G. 1944. *Bull. Johns Hopkins Hosp.* **74,** 85–97.
148. Mackenzie, J. B., Mackenzie, C. G., and McCollum, E. V. 1941. *Science* **94,** 518–9.
149. Malcolm, J. L., and Whitehead, V. I. E. 1944. *Brit. J. Exptl. Path.* **25,** 160–3.
150. Malkiel, S., 1946. *Endocrinology* **38,** 58–9.
151. Mann, C. W. 1945. *J. Psychol.* **20,** 91–9.
152. Marine, D., and Baumann, E. J. 1945a. *Am. J. Physiol.* **144,** 69–73.
153. Marine, D., and Baumann, E. J. 1945b. *Am. J. Physiol.* **144,** 742–6.
154. Marine, D., and Baumann, E. J. 1945c. *Arch. Path.* **39,** 325–30.
155. Marine, D., Baumann, E. J., and Cipra, A. 1929. *Proc. Soc. Exptl. Biol. Med.* **26,** 822–24.
156. Marine, D., Baumann, E. J., Spence, A. W., and Cipra, A. 1932. *Proc. Soc. Exptl. Biol. Med.* **29,** 772–5.
157. May, L. G., Moseley, R. W., and Forbes, J. C. 1946. *Endocrinology* **38,** 147–51.
158. McArthur, J. W., Rawson, R. W., and Means, J. H. 1945. *Ann. Internal Med.* **23,** 915–23.

159. McCarrison, R. 1917. The Thyroid Gland in Health and Disease. Wm. Wood, New York.
160. McCarrison, R. 1933. *Brit. Med. J.* **2,** 671–5.
161. McCarrison, R. 1937. *Brit. Med. J.* **1,** 29–31.
162. McGavack, T. H., Gerl, A. J., Morton, J. H., Vogel, M., and Schwimmer, D. 1945. *J. Clin. Endocrinology* **5,** 259–72.
163. McGavack, T. H., Gerl, A. J., Vogel, M., and Schwimmer, D. 1944. *J. Clin. Endocrinology* **4,** 249–61.
164. McGavack, T. H., and Vogel, M. 1945. *Bull. N. Y. Med. Coll., Flower 5th Ave. Hosp.* **8,** 89–99.
165. McGinty, D. A., and Bywater, W. G. 1945a. *J. Pharmacol.* **84,** 342–57.
166. McGinty, D. A., and Bywater, W. G. 1945b. *J. Pharmacol.* **85,** 129–39.
167. McGinty, D. A., and Sharp, E. A. 1946. *Endocrinology* **39,** 74.
168. McGovran, E. R., and Piquett, P. G. 1943. *J. Econ. Entomol.* **36,** 936.
169. McShan, W. H., Meyer, R. K., and Johansson, D. R. 1946. *Endocrinology* **38,** 152–5.
170. Means, J. H. 1937. The Thyroid and Its Diseases, Lippincott, Philadelphia, p. 331.
171. Meyer, A. E.; Collins, M. B., and Marine, D. 1944. *Proc. Soc. Exptl. Biol. Med.* **55,** 221–2.
172. Meyer, A. E., and Ransom, G. V. 1945. *Endocrinology* **36,** 259–65.
173. Miller, W. H., Roblin, R. O., Jr., and Astwood, E. B. 1945. *J. Am. Chem. Soc.* **67,** 2201–4.
174. Mixner, J. P., Reineke, E. P., and Turner, C. W. 1944. *Endocrinology* **34,** 168–74.
175. Moore, D. H., Levin, L., and Smelser, G. K. 1945. *J. Biol. Chem.* **157,** 723–9.
176. Morton, M. E., and Chaikoff, I. L. 1943. *J. Biol. Chem.* **147,** 1–9.
177. Morton, M. E., Chaikoff, I. L., Reinhardt, W. O., and Anderson, E. 1943. *J. Biol. Chem.* **147,** 757–69.
178. Muhrer, M. E., and Hogan, A. G. 1945. *Proc. Soc. Exptl. Biol. Med.* **60,** 211–2.
179. Nigrelli, R. F., Goldsmith, E. D., and Charipper, H. A. 1946. *Anat. Record Suppl.* **94** 523.
180. Noble, G. K., and Bradley, H. T. 1933. *Biol. Bull.* **64,** 289–98.
181. Paschkis, K. E. 1944. The Medical Clinics of North America. No. Philadelphia, Penna., pp. 1362–75.
182. Paschkis, K. E., Cantarow, A., Hart, W. M., and Rakoff, A. E. 1944. *Proc. Soc. Exptl. Biol. Med.* **57,** 37–9.
183. Paschkis, K. E., Cantarow, A., Rakoff, A. E., and Rothenberg, M. S. 1945. *Endocrinology* **37,** 133–5.
184. Paschkis, K. E., Cantarow, A., Rakoff, A. E., and Tillson, E. K. 1945a. *J. Pharmacol.* **83,** 270–4.
185. Paschkis, K. E., Cantarow, A., Rakoff, A. E., and Tillson, E. K. 1945b. *Science* **102,** 333–4.
186. Paschkis, K. E., Cantarow, A., and Tillson, E. K. 1945. *Proc. Soc. Exptl. Biol. Med.* **60,** 148–52.
187. Paschkis, K. E., Cantarow, A., and Tillson, E. K. 1946. *Federation Proc.* **5,** 79.
188. Phillips, F. S. 1946. *Federation Proc.* **5,** 292–8.
189. Pipes, G. W., and Turner, C. W. 1946. 35th Ann. Meeting Poultry Sci. Assoc.
190. Plummer, H. S. 1923. *J. Am. Med. Assoc.* **80,** 1955.
191. Purves, H. D. 1943. *Brit. J. Exptl. Path.* **24,** 171–3.

192. Raab, W. J. 1944. *J. Pharmacol.* **82,** 330–8.

193. Raab, W. J. 1945. *J. Am. Med. Assoc.* **128,** 249–56.

194. Rawson, R. W., Albert, A., McArthur, J. W., Merrill, P., Lennon, B., and Riddell, C. B. 1946. *Endocrinology* **39,** 74.

195. Rawson, R. W., Cortell, R. E., Peacock, W., and Means, J. H. 1944. *Endocrinology* **35,** 201–2.

196. Rawson, R. W., Evans, R. D., Means, J. H., Peacock, W. C., Lerman, J., and Cortell, R. E. 1944. *J. Clin. Endocrinol.* **4,** 1–11.

197. Rawson, R. W., Graham, R. M., and Riddell, C. B. 1943. *Ann. Internal Med.* **19,** 405–14.

198. Rawson, R. W., Hertz, S., and Means, J. H. 1943. *Ann. Internal Med.* **19,** 829–41.

199. Rawson, R. W., McGinty, D. A., and Peacock, W. 1946. *Endocrinology* **39,** 78.

200. Rawson, R. W., Moore, E. D., Peacock, W., Means, J. H., Cope, O., and Riddell, C. B. 1945. *J. Clin. Investigation* **24,** 869–77.

201. Rawson, R. W., Sterne, G. D., and Aub, J. C. 1942. *Endocrinology* **30,** 240–5.

202. Rawson, R. W., Tannheimer, J. F., and Peacock, W. 1944. *Endocrinology* **34,** 245–53.

202a. Reineke, E. P. 1946. *Vitamins & Hormones* **4,** 207–253.

203. Reineke, E. P., Mixner, J. P., and Turner, C. W. 1945. *Endocrinology* **36,** 64–7.

204. Reineke, E. P., and Turner, C. W. 1945. *Poultry Sci.* **24,** 499–504.

205. Řeřábek, J. 1943. *Biochem. Z.* **314,** 291.

206. Reveno, W. S. 1945. *J. Clin. Endocrinol.* **5,** 403–6.

207. Richter, C. P. 1945. *J. Am. Med. Assoc.* **129,** 927–31.

208. Richter, C. P., and Clisby, K. H. 1941. *Proc. Soc. Exptl. Biol. Med.* **48,** 684–7.

209. Salter, W. T. 1940. The Endocrine Function of Iodine. Harvard Univ. Press, Cambridge, Mass.

210. Salter, W. T., Cortell, R. E., and McKay, E. A. 1945. *J. Pharmacol.* **85,** 310–23.

211. Salter, W. T., and Lerman, J. 1936. *Endocrinology* **20,** 801–8.

212. Schachner, H., Franklin, A. L., and Chaikoff, I. L. 1943. *J. Biol. Chem.* **151,** 191–9.

213. Schachner, H., Franklin, A. L., and Chaikoff, I. L. 1944. *Endocrinology* **34,** 159–67.

214. Schaefer, W. H. 1933. *Proc. Soc. Exptl. Biol. Med.* **30,** 1363–5.

215. Schnetzler, E. E., Andrews, F. N., and Hauge, S. M. 1945. Nov.–Dec. Issue, *U. S. Egg & Poultry Mag.* 1–4.

216. Schultze, A. B., and Turner, C. W. 1944. *Yale J. Biol. Med.* **17,** 269–80.

217. Schultze, A. B., and Turner, C. W. 1945. *Univ. Mo. Agr. Exptl. Sta. Bull.* **392,** 1–89.

218. Segaloff, A. 1944. *Endocrinology* **35,** 134–6.

219. Sharpless, G. R. 1938. *Proc. Soc. Exptl. Biol. Med.* **38,** 166–8.

220. Sharpless, G. R., and Hopson, E. M. 1940. *Endocrinology* **27,** 129–31.

221. Sharpless, G. R., Pearsons, J., and Prato, G. S. 1939. *J. Nutrition* **17,** 545–55.

222. Sloan, M. H., and Shorr, E. 1944. *Science* **99,** 305–7.

223. Smithcors, J. F. 1945. *Proc. Soc. Exptl. Biol. Med.* **59,** 197–200.

224. Sperry, W. M., Jailer, J. W., and Engle, E. T. 1944. *Endocrinology* **35,** 38–48.

225. Taber, E., and Domm, L. V. 1946. *Anat. Record Suppl.* **94,** 22.

226. Taurog, A., Chaikoff, I. L., and Franklin, A. L. 1945. *J. Biol. Chem.* **161,** 537–43.
227. Terry, G. S. 1918. *J. Exptl. Zoöl.* **24,** 567–88.
228. Thomas, O. L. 1944. *Anat. Record* **89,** 461–71.
229. Thompson, W. O., and Thompson, P. K. 1945. *J. Lab. Clin. Med.* **30,** 354.
230. Tipton, S. R., and Nixon, W. L. 1946. *Endocrinology* **39,** 300–6.
231. Turner, C. W. 1946. *Poultry Sci.* **25,** 186–7.
232. VanderLaan, W. P., and Bissell, A. 1946a. *Endocrinology* **38,** 308–14.
233. VanderLaan, W. P., and Bissell, A. 1946b. *Endocrinology* **39,** 157–60.
234 Van Winkle, W., Jr., Hardy, S. M., Hazel, G. R., Hines, D. C., Newcomer, H. S., Sharp, E. A., and Sisk, W. N. 1946. *J. Am. Med. Assoc.* **130,** 343–7.
235. Vogel, M., and McGavack, T. H. 1946. *J. Pharmacol.* **86,** 120–8.
236. Wagner-Jauregg, T., and Schreiber, E. 1944. *Biochem. Z.* **317,** 21–5.
237. Waller, R. K., and Charipper, H. A. 1945. *Am. J. Med. Sci.* **210,** 443–52.
238. Warren, C. O. 1945a. *Am. J. Physiol.* **145,** 71–6.
239. Warren, C. O. 1945b. *Science* **102,** 175–6.
240. Webster, B. 1929. *Bull. Johns Hopkins Hosp.* **45,** 215–31.
241. Webster, B., and Chesney, A. M. 1928. *Bull. Johns Hopkins Hosp.* **43,** 291–308.
242. Webster, B., Clawson, T. A., and Chesney, A. M. 1928. *Bull. Johns Hopkins Hosp.* **43,** 278–90.
243. Westerfeld, W. W., and Lowe, C. 1942. *J. Biol. Chem.* **145,** 463–70.
244. Wilgus, H. S., Jr., Gassner, F. X., Patton, A. R., and Gustavson, R. G. 1941. *J. Nutrition* **22,** 43–52.
245. Williams, R. H. 1944a. *Arch. Internal Med.* **74,** 479–87.
246. Williams, R. H. 1944b. *J. Clin. Endocrinol.* **4,** 385–93.
247. Williams, R. H. 1946. *J. Clin. Endocrinol.* **6,** 1–22.
248. Williams, R. H., and Bissell, G. W. 1943. *New Engl. J. Med.* **229,** 97–108.
249. Williams, R. H., Bissell, G. W., Jandorf, B. J., and Peters, J. B. 1944. *J. Clin. Endocrinol.* **4,** 58–64.
250. Williams, R. H., and Clute, H. M. 1944. *New Engl. J. Med.* **430,** 657–67.
251. Williams, R. H., and Clute, H. M. 1945. *J. Am. Med. Assoc.* **128,** 65–8.
252. Williams, R. H., Clute, H. M., Anglem, T. J., and Kenney, F. R. 1946. *J. Clin. Endocrinol.* **6,** 23–51.
253. Williams, R. H., and Frame, E. G. 1945. *Bull. Johns Hopkins Hosp.* **77,** 314–28.
254. Williams, R. H., Jandorf, B. J., and Kay, G. A. 1944. *J. Lab. Clin. Med.* **29,** 1–8.
255. Williams, R. H., and Kay, G. A. 1944. *J. Clin. Endocrinol.* **4,** 385–93.
256. Williams, R. H., and Kay, G. A. 1945. *Am. J. Physiol.* **143,** 715–22.
257. Williams, R. H., Kay, G. A., and Jandorf, B. J. 1944. *J. Clin. Investigation* **23,** 613–27.
258. Williams, R. H., and Peters, J. B. 1945. *J. Clin. Endocrinol.* **5,** 217–9.
259. Williams, R. H., Weinglass, A. R., Bissell, G. W., and Peters, J. B. 1944. *Endocrinology* **34,** 317–28.
260. Williams, R. H., Weinglass, A. R., and Kay, G. A. 1944. *Am. J. Med. Sci.* **207,** 701–5.
261. Williamson, J. W., Jr., Warren, M. R., and Werner, H. W. 1945. *J. Pharmacol.* **85,** 234–6.
262. Wolff, J., Chaikoff, I. L., Taurog, A., and Rubin, L. 1946. *Endocrinology* **39,** 140–8.

ADDENDUM

During the time this chapter was in press, more than 70 additional articles on the subject of antithyroid agents have been published. The majority of these have been essentially confirmations or extensions of work already described in the present report. Thus, more detailed studies have been made of the effects of thiourea and thiouracil on thyroid and pituitary cytology (Grasso, R. 1946. *Anat. Record* **95,** 365–78; Purves, H. D., and Griesbach, W. E. 1946. *Brit. J. Exptl. Path.* **27,** 170–9; Dawson, A. B. 1947. *Anat. Record Suppl.* **97,** 67). Additional information has been provided concerning the nature and incidence of neoplastic and metastatic thyroid growths in rats and mice under prolonged treatment with thiourea and its derivatives (Gorbman, A. 1946. *Cancer Research* **6,** 492; Crane, A. R., and Payne, R. L. 1946. *Am. J. Path.* **22,** 639–40; Money, W. L. 1946. *Anat. Record Suppl.* **96,** 48–9; Purves, H. D., and Griesbach, W. E. 1946. *Brit. J. Exptl. Path.* **27,** 294–7; 1947. *ibid.* **28,** 46–53).

Body growth, hatchability, edibility, reproductive activity, and feather growth and coloration in fowls under the influence of antithyroid drugs have been subjected to closer scrutiny (Grossowicz, N. 1946. *Proc. Soc. Exptl. Biol. Med.* **63,** 151–2; Glazener, E. W., and Jull, M. A. 1946. *Poultry Sci.* **25,** 236–41; Turner, C. W. *ibid.* **25,** 517–20; Juhn, M., and Jull, M. A. 1946. *Anat. Record Suppl.* **96,** 92–4). Inhibition of amphibian metamorphosis has been utilized as a criterion for determining the effectiveness of a series of antithyroid chemicals (Gasche, P. 1946. *Experientia* **2,** 24–7).

More data have been furnished on the chemical, metabolic and histopathological changes induced in rats and dogs given α-naphthylthiourea (Du Bois, K. P., Holm, L. W., and Doyle, W. L. 1946. *J. Pharmacol.* **87,** 53–62; Chanutin, A., Gjessing, E. C., and Ludewig, S. 1947. *Proc. Soc. Exptl. Biol. Med.* **64,** 174–9; Fitzhugh, O. G., and Nelson, A. A. *ibid.* **64,** 305–10; Dieke, S. H. 1947. *Endocrinology* **40,** 123–36). A variety of factors influencing the resistance of rats to α-naphthylthiourea have also been described (Rogers, P. V. 1946. *Proc. Soc. Exptl. Biol. Med.* **63,** 38–40; Richter, C. P. *ibid.* **63,** 364–72).

Further attempts have been made to elucidate the *modus operandi* of the antithyroid drugs. Evidence is presented in the detailed study by Mackenzie, C. G. (1947. *Endocrinology* **40,** 137–53), which suggests that the antithyroid activity of thiourea, thiouracil and *p*-aminobenzoic acid is due to their ability to reduce iodine to iodide. Sulfaguanidine appears to act through a different mechanism, presumably enzymatic. More data are accumulating in favor of the view that the thioureas operate by combining with iodine (Forminge, P. 1946. *Proc. Nederland. Koninkl. Akad. Wetenschap.* **59,** 484–5; Calvo, J., and Geomine, J. 1946. *Arch. Biochem.* **10,** 531–6; De Nogales, C., and collaborators. 1946. *Anal. Medicina* **33,** 411–70; Albert, A., Rawson, R. W., *et al.* 1947. *Endocrinology* **40,** 299–307).

Additional studies have been made on the correlation of chemical structure and antithyroid activity (Williams, R. H., and Kay, G. A. 1947. *Am. J. Med. Sci.* **213,** 198–205). A new important development in this field is the identification of several structural analogues antagonistic to thyroxine (Woolley, D. W. 1946. *J. Biol. Chem.* **164,** 11–17).

More recent clinical reports have dealt with the incidence of remission and relapse in thyrotoxic patients following discontinuation of treatment with thiouracil and several of its derivatives. The effectiveness and smaller toxicity of propyl and methyl thiouracil have been stressed (Astwood, E. B., and VanderLaan, W. P. 1946. *Ann. Internal Med.* **25,** 813–21; Wilson, A. 1946. *Lancet* **250,** 640–3; Rose, E., and McConnell, J. 1947. *Am. J. Med. Sci.* **213,** 74–80; McGavack, T. H., Gerl, A. J., Vogel, M., and Schutzer, S. *ibid.* **213,** 144–9; Bartels, E. C., *ibid.* **213,** 150–5).

The reader is also referred to the abstracts of papers on the subject of antithyroid agents presented at the recent meetings of the American Society of Zoologists (1946. *Anat. Record Suppl.* **96,** No. 4), American Association of Anatomists (1947. *Anat. Record Suppl.* **97,** No. 3), American Society of Physiologists (1947. *Federation Proc.* **6,** No. 1) and the N. Y. Academy of Sciences Conference on Thyroid Function, January, 1947. The papers on antithyroid drugs by De Nogales and his collaborators (1946. *Anal. Medicina,* **33,** 411-70) will be found useful for experimental and clinical foreign references on the subject.

The Use of Androgens in Women[1,2,3]

By ANNE C. CARTER, EUGENE J. COHEN, AND EPHRAIM SHORR

CONTENTS

Page

- I. Introduction . . . 318
- II. Relative Potencies and Modes of Administration of Androgens . . . 319
- III. Biological Effects of Androgens in Women . . . 325
  - 1. Anterior Pituitary . . . 326
  - 2. Ovary . . . 327
  - 3. Uterus . . . 329
    - a. Endometrium . . . 329
    - b. Myometrium . . . 330
    - c. Fallopian Tubes . . . 331
  - 4. Vaginal Epithelium and Secretions . . . 331
  - 5. Menstruation . . . 333
  - 6. Breast . . . 334
  - 7. Virilizing Effects . . . 334
    - a. Acne . . . 334
    - b. Hypertrichosis . . . 335
    - c. Vulva and Clitoris . . . 335
    - d. Voice . . . 336
  - 8. Urinary Tract . . . 336
    - a. Kidney . . . 336
    - b. Bladder . . . 337
  - 9. Urinary 17-Ketosteroids . . . 337
  - 10. Metabolic Effects . . . 338
  - 11. Vascular Effects . . . 338
  - 12. Psychobiological Effects . . . 339
- IV. The Clinical Use of Androgens . . . 339
  - 1. Uterine Bleeding . . . 339
    - a. Functional . . . 339
    - b. In Association with Myoma . . . 345
  - 2. Pelvic Disorders . . . 347
    - a. Endometriosis . . . 347
    - b. Pelvic Inflammatory Disease . . . 349
    - c. Endocervicitis . . . 350
    - d. Malignancy of the Genital Tract . . . 350
    - e. Functional Urinary Disturbances . . . 350

---

[1] Investigations of the writers reported in this article were aided by grants to Dr. Shorr from the Josiah Macy, Jr., Foundation and the Schering Corporation.

[2] The steroidal hormones used by the authors in their studies were made available through the generous cooperation of Dr. Erwin Schwenk of the Schering Corporation.

[3] Grateful acknowledgment is made to Doctor H. J. Stander for the assistance provided for the authors' studies by the Department of Obstetrics and Gynecology of The New York Hospital and the Cornell University Medical College.

Page
3. Dysmenorrhea 351
4. Premenstrual Tension 356
5. The Menopausal Syndrome 358
6. Libido 361
7. Functional and Pathological Disorders of the Breast 364
    a. Breast Pain and Benign Lesions 364
    b. The Painful Postpartum Breast and Lactation 369
    c. Breast Carcinoma: Primary and Metastatic 371
8. Disorders Related to Pregnancy 372
    a. Nausea and Vomiting 372
    b. After-Pains 374
9. Metabolic and Endocrine Disorders 374
    a. Cushing's Syndrome 374
    b. Postmenopausal Osteoporosis 376
    c. Simmonds Disease 376
    d. Acromegaly 376
    e. Pituitary Dwarfism 377
    f. Addison's Disease 377
    g. Graves' Disease 378
    h. Muscular Dystrophy 379
    i. Debilitated States 380
10. Miscellaneous 380
    a. Diseases Characterized by Menstrual Exacerbations 380
    b. Diseases with a High Incidence in Women 380
    c. Disorders of the Skin 381
    d. Psychiatric Disorders 381
V. Conclusions 382
References 383

I. INTRODUCTION

For approximately a decade androgens have been utilized as therapeutic agents in women. The too rigid early concept of androgens as male hormones seemed at first to lend merely an academic interest to their application to women; but with the growing awareness that they were capable of profoundly altering the sexual physiology of women their potential value as therapeutic agents began to be appreciated. In the intervening decade they have been widely used in the management of a variety of derangements of female sex physiology and more recently for numerous metabolic disorders. The literature which has accumulated is now sufficiently extensive to permit a tentative evaluation of the present status of androgens as therapeutic agents in women. Such an evaluation must depend for its validity on the extent to which therapeutic studies have been reinforced with objective criteria. Clinical studies provide much less objective information than is made possible by animal experimentation, particularly those dealing with the many clinical conditions in which the presenting phenomena are largely subjective. In their appraisal of the literature, the reviewers have had to deal with a number of clinical applications in which their own experience has been limited. Where judgments as to therapeutic effectiveness appear to be somewhat arbitrary in the light of published evidence, they have been based on unpublished observations either in their own or other laboratories, with which they are familiar. The review is to some degree incomplete because of the inaccessibility of some of the foreign literature of the war years. It is designed to be complementary to that of Kochakian in *Vitamins & Hormones* (1946) on the metabolic effects of androgens and to the companion review in this volume by Heller and Maddock (1947) on their clinical use in men. The major disorders in women for which androgens have been employed are prefaced by a brief consideration of the underlying pathophysiology. This background should assist the reader in forming his own judgments as to the therapeutic claims as well as the mechanisms by which androgens may exert their effects, and should call attention to those broad areas in which a valid basis for the therapeutic use of androgens remains to be provided. The purpose and scope of this review of necessity restricts consideration of the extensive experimental studies in animals to the limited number which are particularly relevant to, and essential for, understanding the effects of androgens in women.

II. RELATIVE POTENCIES AND MODES OF ADMINISTRATION OF ANDROGENS

Although a number of naturally occurring and synthetic steroids containing a cyclopentenophenanthrene nucleus have been shown by animal experimentation to possess androgenic properties, a consideration of their relative potency and availability has, except for specialized investigations, confined their clinical use to four: free testosterone, its propionic and acetic acid esters, and 17-methyl testosterone. For brevity's sake, these compounds will usually be designated in this review by the following abbreviations: T for free testosterone, TP for testosterone propionate, TAc for testosterone acetate and MT for 17-methyl testosterone. Since TP and TAc are usually administered intramuscularly, and MT orally, these modes of administration are to be assumed for these compounds, unless otherwise stated.

Inasmuch as the results of the bioassay of androgens in animals vary widely with the different species used and the biological criteria employed, it has been necessary to turn to their bioassay in the human subject for the evaluation of the relative potencies of the several compounds in clinical use. For this purpose, as with animal assays, a variety of criteria have been utilized, which may be classified under the following headings:

Subjective. This category includes the effects reported to be induced on a wide variety of symptoms in both sexes, such as flushes, nervousness, emotional instability, mood, strength, premenstrual tension, dysmenorrhea, libido and potency, to mention those most commonly elicited. These criteria are recognized to be potentially untrustworthy and to be used with caution as a basis for quantitative assay; nevertheless, they may, under certain circumstances, comprise the only available yardstick and, in experienced hands, can yield valuable information.

Biological. These comprise the estimation, in men, of the extent of growth and development of the sexual structures such as the external genitalia, prostate and seminal vesicles, the rate of growth of the beard, axillary and pubic hair, the appearance of acne and voice changes, the increase in weight and muscle bulk, and the maturation of the bony skeleton. In women, besides the virilizing effects, which include the enlargement of the clitoris, the action of androgens is indicated by the suppression of the menstrual and vaginal cycle, by alterations in the endometrial pattern, and the suppression of postpartum breast engorgement and lactation in the non-nursing mother. Even with these more reliable criteria, the interpretation of the results may be complicated by factors such as individual differences in end-organ responsiveness, alterations in sensitivity to the hormone in the same individual at different stages of treatment, as well as the frequency with which sexual development, once initiated in the young sexually undeveloped male, may proceed spontaneously.

Biochemical. To this expanding category belong such measurable changes as those induced by androgens on nitrogen metabolism, creatinuria, urinary citric acid, and the excretion of gonadotropins and androgens, as determined by bioassay or the colorimetric estimation of 17-ketosteroids. Biochemical studies are particularly exacting as regards the maintenance of uniform conditions throughout the period of study, and the necessity for prolonged control and experimental periods. Even under ideal conditions, the accuracy and specificity of the hormonal assays leave much to be desired.

Whatever the criteria, the methods employed should be consistent with recognized principles of bioassay. A series should be large enough to acquire statistical significance. In smaller series, comparative studies may become valid only when they are carried out on the same individual under uniform conditions, and the results are regularly reproducible. Finally, the large number and variety of symptomatic, biological and biochemical effects produced by the androgens make it hazardous to assume that activity ratios obtained with one index are valid for any other.

Inasmuch as the relative potency of these compounds is conditioned not only by inherent androgenicity but also by the mode of administration, the information available in the literature will be presented in relation to the mode of administration.

a. Parenteral. The early demonstration in the rat (Miescher, Wettstein and Tschopp, 1936; Miescher *et al.*, 1937; Kochakian, 1938) that the short-chain fatty acid esters of testosterone exerted a more pronounced and prolonged effect on seminal vesicle and prostate weight than free testosterone, promptly led to the adoption of the propionic, and, less generally, the acetic acid ester for clinical use by the parenteral route. This is undoubtedly responsible for the paucity of information as to the relative potencies of testosterone and its esters in man.

Menstruum. In common with other steroidal hormones, the androgens are dissolved in a vegetable oil, usually peanut or sesame, for parenteral administration. Experience with a recently introduced aqueous suspension of testosterone is still too meager to permit a comparison with oily solutions. It was, however, effective in initiating sexual development in a young hypogonadal male, abolishing symptoms of the male menopause (Severinghaus and Sikkema, 1946), and controlling premenstrual tension (Freed, 1946).

Site of Injection. Although this is usually intramuscular, it has been stated that testosterone propionate is 1.5–2 times as effective when injected subcutaneously (Abarbanel, 1940a). This claim, based on symptomatic effects in dysmenorrhea and the menopausal syndrome in women, still awaits confirmation.

Testosterone Propionate (TP) versus Testosterone (T) and Methyl Testosterone (MT). Rat assays have shown that TP induces a significantly greater increase in seminal vesicle and prostate weight than T or MT, the latter compounds being of equal potency (Greene *et al.*, 1942). Nitrogen balance experiments by Wilkins and Fleischmann (1946) on two sexually retarded adolescent males indicate that T and TP exert an approximately equivalent nitrogen sparing action. No other information is available as to the relative effectiveness of intramuscular TP, T and MT in the same individual or using the same index.

b. Oral. Following the pioneer observations of Miescher and Tschopp (1938) which served to establish the superiority of MT over TP and T when given orally to rats, MT became the oral androgen of choice for clinical use. As a consequence, there is little or no reliable information as to the relative oral potencies of these three compounds in man. It has been claimed, on the basis of symptomatic relief of dysmenorrhea, that the administration of bile salts with TP given orally enhances its unstated basal potency to $\frac{1}{7}$ that of TP intramuscularly (Abarbanel, 1940a).

Methyl Testosterone (oral) versus Testosterone Propionate (i.m.). A number of studies have been carried out with a view to establishing the activity ratios of MT given orally and injected TP. These have been assembled in Table I, together with a brief description of the criteria employed. It should be pointed out that in most of these studies, oral MT has been given daily, usually in divided doses, whereas TP has been injected at less frequent intervals, usually 2–3 times weekly. To judge

TABLE I

Relative Potencies of Methyl Testosterone and Testosterone Propionate

Author	Activity ratio MT(o):TP(i.m.)	Sex	No. of patients	Criteria
McCullagh (1940).....	3–4:1	Males	19?	Maintenance of development of secondary sexual characteristics and sexual behavior in pituitary hypogonadism.
Byron and Katzen (1941)..............	3.5:1	Males	2	Ditto.
Escamilla and Lisser (1941).............	2–4:1	Males	3	Ditto.
Salmon (1941)........	2–3:1	Females	46	Suppression of menstruation and vaginal smear cycle. No comparative studies on the same patients.
Berlind (1941)........	3:1	Females	?	Control of menorrhagia and suppression of lactation.
Duffy and Corsaro (1941)	2–3:1	Females	25?	Relief of breast engorgement and suppression of lactation in non-nursing postpartum women.
Talbot, Butler and MacLachlan (1943)..	3.5:1	Female	1	Induction of positive nitrogen balance in Addison's disease.
Kinsell, Hertz and Reifenstein (1944)...	4:1	Females	2	Induction of positive nitrogen balance in thyrotoxicosis.
Wilkins and Fleischmann (1946)........	1:1	Males	2	Induction of positive nitrogen balance in young sexually retarded males.

from vaginal smear observations (Shorr—unpublished data) on the loss of efficiency which results from increasing the intervals between injections of estrogens, a more accurate evaluation of the relative potencies of oral MT and intramuscular TP would require the daily administration of minimal effective doses of the latter.

Methyl Testosterone (*oral*) *versus Methyl Testosterone* (*i.m.*). The creatinuria induced by the oral administration of 20 mg. MT was stated to be duplicated by the intramuscular injection of an equal amount in oil (Wilkins and Fleischmann, 1945).

c. Sublingual. Since the very considerable loss of the activity of natural steroidal hormones given orally is thought to be due in large measure to inactivation or excretion by the liver, the sublingual route has been resorted to in an effort to by-pass this organ. The extensive vascular bed of the mouth and particularly of the under surface of the tongue, as well as its superficial distribution, should favor the rapid absorption of these hormones into the peripheral circulation. Two types of preparations have been used for this purpose, small compressed tablets of MT, and solutions of T, TP and MT in propylene glycol. The technic of administration requires a minimum of salivation and swallowing during the period of absorption, a matter of 2–5 minutes with solutions in propylene glycol and 20 minutes or more with the compressed tablets. These conditions for uniform absorption would, *a priori*, seem to render the sublingual route less reliable for quantitative assay than other methods. The most extensive comparative study is that of Lisser, Escamilla and Curtis (1942) who based their evaluation, in part, on subjective criteria, in part, on the effects induced on genital development and other secondary sexual characteristics in hypogonadal males. On a weight basis, they rate T > MT = TP, the superiority of T, however, being slight. Sublingual T and oral MT were reported to be equally effective on a weight basis. Margolese (1944) compared the relative effectiveness of oral MT and MT tablets given sublingually in inhibiting bleeding when administered simultaneously with stilbestrol; he arrived at the inexplicably high ratio of 5:1 in favor of the sublingual route.

d. Percutaneous. Percutaneous absorption of T, TP and MT incorporated in an ointment base, or better, in alcohol, was shown to take place in the rat (Greene *et al.*, 1942); MT and T were equally active and more effective than TP. The clinical use of this mode of administration has been limited, very likely because of its inefficiency and for aesthetic reasons; hence, there are no good data by which to judge the relative merits of this route. From symptomatic effects on dysmenorrhea and premenstrual tension, Abarbanel (1940a) would assign to TP in an ointment base $\frac{1}{6}$, and in sesame oil $\frac{1}{3}$, the activity of TP given subcutaneously.

e. Pellets. The introduction by Deansley and Parkes (1937) of the pellet implantation technic as a means of administering steroidal hormones to animals was soon followed by its widespread adoption for the same purpose in man. Subsequent experience has served to establish it as the most economical method for providing continuous absorption and prolonged effectiveness. Certain general principles have evolved from the study of the rate of absorption from pellets in animals and man. This has been found to be determined by the size, shape, and degree of compression of the pellet. Both disc-shaped and cylindrical pellets have been used: the former appear to insure a more constant rate of absorption (Emmens, 1941) but their implantation entails a minor surgical procedure; the latter are more convenient since they can be introduced by means of a trocar. In time the pellet acquires a fibrous capsule, which was formerly held responsible for the observed decrease in absorption with time (Howard and Jewett, 1942; Greenblatt and Hair, 1942). Later studies have minimized this influence and appear to have established the principle that the absorption rate for each steroidal hormone is a function of surface area (Shimkin *et al.*, 1944; Cowie and Folley, 1946). Animal studies have shown that the absorption rate from pellets of different androgens may vary; T and MT were absorbed with equal rapidity, TP more slowly (Emmens, 1941; Kochakian, 1942; Forbes, 1941). Comparative studies in man of the relative efficiencies of pellets of these androgens, in relation to each other and to intramuscular TP and oral MT are complicated by variations in the size and shape of the pellets employed. They have, however, served to establish the economy of the method as well as its therapeutic effectiveness. The studies of Vest and Howard (1939) in men, and of Greenblatt *et al.* (Greenblatt and Wilcox, 1941; Greenblatt and Hair, 1942; Greenblatt 1943a) in women, have provided some basis for estimating the rate of absorption in relation to pellet size. The first clinical study of the effects of TP pellets in women by Loeser (1940) was soon followed by similar studies by other workers. Only those which bear on the relative effectiveness of this mode of administration will be considered at this time. Among the most extensive are those of Greenblatt (1943a) on more than 100 women who received pellets of TP implanted subfascially beneath the rectus sheath, in amounts ranging from 25 to 400 mg. It is of considerable interest that Greenblatt observed no virilizing effects in women receiving amounts which are effective in initiating and maintaining sexual development in hypogonadal males.

Methyl Testosterone Pellets versus Testosterone Propionate Intramuscularly. Biskind, Escamilla and Lisser (1941), relying on sexual behavior and the maintenance of secondary sexual structures in hypogonadal males, have compared the androgenic activity of MT pellets with TP i.m. In most instances, very small pellets weighing from 5 to 9 mg. were employed with a total dosage of from 100 to 180 mg.; in 3 cases, 200 mg. pellets were implanted; the total dose ranged from 600 to 800 mg. In these 3 cases, it was possible to compare the duration of the virilizing effects of the large and small pellets; in every instance, the duration of effectiveness of the larger pellets was less than might be anticipated from the increase in the amount of hormone implanted. The smaller pellets of MT were 5–6 times as effective as TP i.m., the latter preparation being administered 3 times weekly. The possibility that this superiority might be lessened by utilizing for comparison the daily minimal requirements of TP i.m. has been broached above.

Methyl Testosterone Pellets versus Methyl Testosterone Orally. The smaller pellets of MT were stated to be 10 times, the larger, 4 times, as effective as MT taken orally.

Summary. Testosterone, testosterone propionate and methyl testosterone in appropriate amounts are capable of exerting a variety of androgenic effects when administered either parenterally in oil or aqueous suspension, in the form of pellets, and when given orally, sublingually or by inunction. The most economical utilization is achieved by pellet implantation, the most convenient by the oral route. Due in part to the variety of indices employed and to difficulties inherent in any human bioassay, the present literature does not permit an entirely satisfactory quantitative evaluation of the relative activities of T, TP and MT by these various routes. The superiority of MT by the oral route and TP in oil for intramuscular administration appears to be well established. No clear-cut advantage exists between MT, TP and T for pellet implantation. The inefficiency and inconvenience of the percutaneous method has served to limit its use. In the absence of any evident advantages of the sublingual route, the oral administration of MT has been generally favored. The question of dosage requirements will be discussed in relation to the specific conditions in women for which androgens have been employed.

III. Biological Effects of Androgens in Women

It will facilitate the evaluation of the clinical use of androgens to review briefly what is known about their specific actions in women. These consist in the effects, both direct and indirect, which they exert on the physiology and histology of the sexual apparatus, on various metabolic functions and on vasomotor, psychological and emotional phenomena. Since the metabolic effects of androgens have already been reviewed in *Vitamins & Hormones* by Kochakian (1946), their discussion will be limited to their application to specific diseases. Certain other actions, such as those which may be exerted on the cardiovascular system and which have been studied chiefly in males are dealt with by Heller and Maddock in this volume. The stimulus for the clinical use of androgens in women was primarily provided by animal studies which revealed their profound effect on the function and structure of the female sexual apparatus.

1. Anterior Pituitary

Castration in animals is followed by typical histological changes in the anterior pituitary (Severinghaus, 1939) and an enhancement of its gonadotropic activity. These histological changes can be repaired or prevented in both sexes by the administration of androgens, with a concomitant reduction of gonadotropic secretory activity to normal levels.

In man, information as to the influence of androgens on pituitary gonadotropic activity is limited to excretion studies and to the phenomena secondary to the action of gonadotropins on the gonads. The menopausal syndrome in women and certain male hypogonadal states are accompanied by heightened urinary excretion of a gonadotropin which exerts a predominantly follicle stimulating effect in animals and which will be referred to as FSH. A number of investigators have shown that the urinary excretion of FSH by menopausal women can be reduced to normal levels by appropriate amounts of androgens (Salmon, 1937; Laroche *et al.*, 1938; Shorr *et al.*, 1938; Nathanson and Towne, 1939; Rothermich and Foltz, 1940; Loeser, 1940). Because of the variety of analytical procedures employed and the inherent inadequacies of current methods for the estimation of gonadotropic activity, only semiquantitative inferences can be drawn concerning the relationship between androgen dosage and the extent of reduction in urinary FSH. Complete suppression of urinary FSH resulted from the administration of 815 mg. TP to a menopausal woman over a period of 27 days, with an average daily dose of 30 mg. (Salmon, 1937). Significant reductions likewise followed injections of 25–50 mg. of TP or TAc at 2–3-day intervals for 35–60 days; whereas urinary FSH excretion was unaffected and in some instances increased when the amounts were reduced to 5–10 mg. and given at 2–4 day intervals for 21–28 days (Laroche *et al.*, 1938).

The relation of these results to the influence of androgens on gonadotropic activity of menstruating women is still somewhat uncertain since studies of their effects on gonadotropic activity are largely, if not entirely, confined to FSH excretion in the menopausal group, whereas two, and possibly three, pituitary factors are involved in the regulation of normal ovarian function. The possibility should be borne in mind that each of these factors may have a different threshold of susceptibility to the androgenic hormones. However, the studies on menopausal women have established a direct effect of androgens on the gonadotropic activity of the pituitary with respect to FSH.

2. *Ovary*

There is no evidence of a direct effect on the ovary in either experimental animals (Selye, 1941; Huffman and Bos, 1940) or man; such changes as have been observed in ovarian histology and function must, for the present, be regarded as secondary to the depression of pituitary gonadotropic activity. Ovaries from mature animals injected with androgens are devoid of corpora hemorrhagica or recent corpora lutea; atretic follicles are present and the primordial follicles appear to be unaltered (Huffman and Bos, 1940). When 300 mg. or more of TP are injected into normal menstruating women for a period of 3–4 weeks, starting with the onset of menstruation, the ovaries contain no maturing follicles or current corpora lutea (Geist *et al.*, 1940a; Huffman, 1940; Salmon *et al.*, 1941b). The conclusion that these amounts of androgens are capable of suppressing both follicle stimulating and luteinizing pituitary hormones is complicated by the uncertainty as to whether or not follicular growth had taken place and had subsequently receded. This uncertainty might be resolved by concomitant studies of urinary estrogen excretion concerning which there is little available information. The possibility of differential effects of androgens on the several pituitary gonadotropic factors will be discussed in relation to the endometrium. The secondary effects of androgens on the ovary are of a temporary character; this is evident from the subsequent resumption of normal menstruation and the frequency with which pregnancies have followed shortly after discontinuation of treatment (Mazer and Mazer, 1939, 1940; Berlind, 1941; Salmon *et al.*, 1941a; Simon, 1946; Shorr, unpublished data).

Confirmatory evidence of the suppressive effect of androgens on corpus luteum formation is provided by the absence of pregnandiol excretion when large doses of androgens are administered during the first half of the cycle. In subjects given TP in amounts totaling 600 mg. during the first 14 days or 1100 mg. during the first 21 days of the menstrual cycle, no current corpora lutea were observed at operation (Salmon *et al.*, 1941b; Salmon, 1941). On the other hand, these investigators observed the excretion of normal amounts of pregnandiol when as much as 1165 mg. of TP were administered from the 10th to the 21st day of the menstrual cycle. Hamblen *et al.* (1941) observed no depression of pregnandiol excretion following the injection of a total of 108 mg. TP between the 10th and 16th day of the cycle. It may be concluded that the suppression of pregnandiol excretion requires the administration of large amounts of androgen during the first half of the cycle and that large doses are without effect when given during the second half.

The influence of androgens on the secretory activity of the Graafian follicle, as contrasted with corpus luteum, has, despite its importance, received little attention. Taylor *et al.* (1943) observed either no change or a slight reduction in total estrogen excretion during a menstrual cycle in which a total of 200 mg. of TP were given from day 8 to 14; when given after ovulation, from day 15 to 21, there was a rise in estrogen excretion during the second half of the cycle. The evaluation of these findings is complicated by the spontaneous variation in estrogen excretion of the same subject during control cycles. Shorr, Papanicolaou and Stimmel (1938, and unpublished data) studied estrogen excretion in a young menstruating subject given 25 mg. TP daily for a period of 25 days beginning on day 14. Estrogen excretion during the first cycle showed the usual high mid-menstrual peak, a well sustained excretion rate during the second half of the cycle while TP was being administered, and the expected fall to menstrual levels on day 22 when an early bleeding occurred. In the following cycle, during the period in which the hormone was being administered daily, the urinary estrogen reached values on days 11 through 14 identical with the mid-menstrual values of the previous cycle. This peak was followed, however, by an abrupt fall which contrasted with the well sustained excretion of the second half of the previous cycle. The subsequent menstruation occurred 7 days after the last injection of TP, 34 days after the previous menstrual period. This isolated study would suggest that follicular secretory activity, with estrogen production, can occur during the administration, by the regime described above, of large amounts of TP throughout the first half of the menstrual cycle. However, it requires confirmation in view of its significance for the analysis of the manner in which androgens exert their suppressive effects on ovarian function through their action on the anterior pituitary gonadotropins.

The remaining studies on the influence of androgens on estrogen excretion have been conducted in male or female menopausal castrates for the purpose of ascertaining whether TP undergoes transformation into estrogens in the organism (Steinach and Kun, 1937; Hoskins *et al.*, 1939; Dorfman and Hamilton, 1939; Nathanson and Towne, 1939; Callow *et al.*, 1939; Laroche *et al.*, 1939). The results were equivocal and irregular, and such increases as were reported were small and quantitatively insignificant in comparison to the amounts of androgens administered.

3. *Uterus*

a. Endometrium. The effects exerted on the endometrium of normally menstruating women are dependent on the amounts given, the duration of treatment, the phase of the cycle during which androgens are administered and on individual susceptibility. When 200 mg. or less of TP are given per month, or TP pellets totaling up to 400 mg. implanted (Greenblatt, 1943a), no effects have been observed on the normal sequence and pattern of endometrial development (Mazer and Mazer, 1939, 1940; Salmon *et al.*, 1939; Geist *et al.*, 1940; Sturgis *et al.*, 1940; Hamblen *et al.*, 1941).

However, the monthly administration of 300 mg. or more of TP almost invariably results in characteristic alterations of the endometrial picture, such as would be anticipated from the changes previously described as taking place in ovarian secretory function under these conditions (Loeser, 1938a; Foss, 1938; Gaines *et al.*, 1938; Geist *et al.*, 1938a; Greenhill and Freed, 1939; Huffman, 1940). When the administration was begun early in the first half of the menstrual cycle and endometrial biopsies obtained at the end of the 3rd or 4th week of continuous treatment, the secretory phase was found to have been suppressed (Geist *et al.*, 1940b). The endometrium may show either a fairly characteristic proliferative pattern or exhibit varying degrees of regression ranging from hypoplasia to atrophy. If injections are deferred until after ovulation, the secretory endometrial pattern for that cycle is unaffected.

These results may be considered in the light of what is known of the relation of pituitary gonadotropic factors to follicular growth, corpus luteum formation and the elaboration of estrogens and progesterone. The present concept, based largely on experiments on the hypophysectomized rat, may be briefly stated. The specific effect of FSH is held to be the stimulation of follicular growth, estrogen production by the follicle requiring the complementary action of small amounts of LH. The increasing estrogen formation associated with follicular maturation reduces FSH formation and leads to a preponderance of LH. This alteration in the ratio of FSH and LH results in the rupture of the follicle and its transformation to a corpus luteum whose persistence and secretion of progesterone are dependent upon the elaboration of a pituitary luteotropic factor (Fevold, 1944).

The suppression of the secretory endometrium and pregnandiol excretion and the usual presence of a proliferative endometrium following the administration of large amounts of androgen during the first half of the cycle would suggest that the pituitary factors responsible for corpus luteum transformation and hormonal function are more sensitive to androgens than those concerned with follicular growth and estrogen production. The failure of large amounts of androgens given during the second half of the cycle to interfere with the luteal phase further suggests that once the production of these pituitary factors has taken place they are resistant to the influence of androgens. From the present meager data it would be unwise to attempt too specific an interpretation of these phenomena, although the inference is permissible that the androgens may exert their effect on ovarian function by causing a derangement in a very subtle chain of reactions between the several pituitary gonadotropic factors and the ovarian hormones. The time sequence and the ratios of hormonal factors essential for the normal ovulatory cycle appear from animal experiments to be so delicate that any disturbance in this chain-reaction might lead to an inhibition of corpus luteum development and progesterone production. Much more information is needed, particularly as to the effects of androgens on the pituitary factors governing the development of the Graafian follicle.

The relation of the endometrial hypoplasia and atrophy, which follows prolonged administration of large amounts of androgens, to alterations in ovarian and pituitary secretory activity, is further complicated by the possible neutralizing effects which androgens may exert on the endometrial proliferation induced by estrogens. Such a direct neutralization by androgens of the effects of estrogens on the vaginal mucosa has been observed and will be discussed later (Shorr, Papanicolaou and Stimmel, 1938). Direct evidence of a similar effect on the human endometrium is lacking, but Markee (1940) observed in ocular endometrial implants in the monkey a direct neutralizing effect by androgens on the growth of the endometrium despite the concomitant administration of estrogens.[4]

One may conclude that androgens interfere with pituitary gonadotropic function so as eventually to suppress the development and secretory activity of the corpus luteum; that their influence on follicular growth requires clarification; and that these effects may be exerted through derangement of a complex series of pituitary-ovarian interactions essential for the development of a normal ovulatory cycle. The degree to which the endometrial pattern is influenced by the neutralizing effects of androgens requires further study.

b. Myometrium. Attention was called to the possible effects of androgens on the human myometrium by the observation of Robson (1937b) that TP inhibited rhythmic motility of the uterus of ovariectomized rabbits primed with estrogen. The interest in this observation lies in the potential value of androgens in the management of essential dysmenorrhea. Information is meager as to the action of androgens on human uterine contractions. Wilson and Kurzrok (1940a) using the intra-uterine balloon method observed the development of the typical luteal contraction pattern following the administration of TP in primary amenorrhea after a total of 500 mg. had been given in amounts of 50 mg. twice weekly. The injection of 100 mg. of TP during the second half of the menstrual cycle to a single dysmenorrheic subject induced no alterations in the uterine contraction pattern during the subsequent menstruation (Bickers, 1941, 1942). The available information is obviously inadequate to permit evaluation of the action of androgens on the myometrium.

---

[4] Since this was written, the neutralization by androgens of the effects of estrogens on the endometrium has been observed in two patients (Carter and Shorr, unpublished data).

c. Fallopian Tubes. Ring segments of fallopian tubes of the pig (Seckinger, 1923) and rhesus monkey (Seckinger and Corner, 1923) exhibit spontaneous contractions *in vivo* characteristic of the phases of the ovarian cycle. During the ovulatory phase, contractions are of a rapid undulatory type; in the inter-estrus phase, they are slower, regular and of greater amplitude. There is little information as to the contraction pattern of the fallopian tubes in normal menstruating women. Menopausal women studied by the trans-uterine tubal insufflation method of Rubin showed a marked diminution in tubal contractions which could be restored to the mid-menstrual pattern by the administration of estrogens (Geist *et al.*, 1938b). When 50 mg. of TP were injected 3 times weekly to menstruating women, complete inhibition of tubal contractions was observed after 500 mg. had been administered (Geist *et al.*, 1939). These results would indicate an opposite effect of androgens on tubal contraction to that reported by Wilson and Kurzrok (1940a) for uterine contractility.

4. Vaginal Epithelium and Secretions

The vaginal mucosa and secretions have been shown to undergo a typical sequence of cytological changes in relation to the different phases of the human menstrual cycle (Papanicolaou, 1933; de Allende, Shorr and Hartman, 1943) which can be duplicated in menopausal castrates by the administration of estrogens (Papanicolaou and Shorr, 1936) and estrogen in combination with progesterone (Shorr, 1940a; Shorr and Cohen, 1940). The influence of androgens on the human vaginal cycle was studied by Papanicolaou, Ripley and Shorr (1938, 1939). TP was administered for a period of 31 days beginning with day 12 to a young woman with regular menstrual cycles. A total of 775 mg. were given over this period in doses of 25–75 mg. every other day. The next menses appeared early, on day 22. By the 6th or 7th day of this cycle (14–15 days from the beginning of treatment) early regressive changes were observed in the vaginal smear which progressed to the development of a typical atrophic smear picture on day 11, the 19th day of treatment. This type of smear persisted for 5 days after discontinuation of therapy; thereafter the vaginal secretions gradually resumed their normal cytological characteristics with the return of menstruation 28 days after the last injection of TP. Because of the simplicity of the vaginal smear method, these investigators suggested that the study of the vaginal secretions might provide a convenient method for guiding therapy with androgens, particularly when the objective is the suppression of the menstrual cycle. The suppressive effect of androgens on the vaginal cycle was subsequently confirmed by Salmon *et al.* (1939) and others (Rothermich, 1939; Greenhill and Freed, 1939; Huffman, 1940; Berlind, 1941).

It was not clear from these studies whether the atrophy of the vaginal mucosa was merely a reflection of the suppression of ovarian hormonal function by androgens or whether it might be due in part to their neutralization of the action of estrogens on the vaginal epithelium, which had been observed by Robson (1937a) in ovariectomized mice. With this in mind, Shorr, Papanicolaou and Stimmel (1938) administered estradiol benzoate to menopausal castrates in amounts sufficient to induce a fully cornified smear. At this stage injections of estrogen were continued and TP given concurrently in 25–50 mg. amounts daily for periods of 17–34 days. The cornified smear previously induced, regressed to the original menopausal atrophic type, and vaginal biopsies showed a loss of the cornified zone which had been previously developed under treatment with estradiol benzoate alone. The neutralizing ratio of TP to estradiol benzoate was 50:1 on a weight basis. In 5 menopausal subjects TP was given alone in 25 mg. amounts daily with no change in the smear picture or the vaginal epithelium, which remained menopausal throughout.

These findings are of interest in view of the reports of several other investigators that androgens may induce estrogenic smears in menopausal subjects. Thus, Salmon (1937) reported that TP in doses of 10–50 mg. daily converted a menopausal to an estrous smear in a surgical castrate after 465 mg. had been given. This author (Salmon, 1941) attempts to reconcile these discrepant results on the basis of dosage level, in view of his subsequent findings that only 8 of 43 menopausal women developed follicular smears following TP, the positive responses being limited to those receiving 50–100 mg. daily for periods of at least 3 weeks. However, other investigators reported the development of estrogenic smears in menopausal women with much smaller amounts of TP (Nathanson and Towne, 1939; Greenblatt, 1943a; Abel, 1945) and of MT (Bolger, 1946).

On the other hand, Herrmann, Adair, and Woodward (1947) observed the invariable induction of atrophic smears following massive doses of TP for several months to menopausal women with metastatic carcinoma. Furthermore, the development of a cornified smear with androgens is contrary to the universal experience that large doses of androgens which suppress menstruation invariably lead to pronounced regressive changes in the vaginal smear.

A number of factors may complicate the evaluation of the vaginal smear and be responsible for these occasional instances where cornified smears were believed to have been induced by androgens. Inadvertent drying of the smear before fixation, the failure to use differential stains and the irritative cornification induced by *Trichomonas* infections are most commonly responsible for errors in interpretation. One additional complication is the occurrence of occasional spontaneous estrogenic activity in menopausal subjects, which might overcome the neutralizing effects of the relatively small doses of androgens in several of the studies cited.

5. Menstruation

The ultimate effects of androgens on the pituitary regulation of ovarian function are expressed in a variety of disturbances of overt menstruation. Complete suppression of menstruation in primates was first observed by Zuckerman (1937) and Hartman (1937). Shortly thereafter a similar suppression of menstruation was reported by Loeser (1938a) in women with menorrhagia and by Papanicolaou, Ripley and Shorr (1938, 1939) in normal menstruating women. These observations were soon confirmed (Gaines *et al.*, 1938; Foss, 1938) and reinforced with information concerning the associated endometrial and ovarian changes.

The extent of the influence of androgens on menstrual bleeding varies from alterations in regularity, duration, and amount of flow, to complete suppression, and are determined by the amount, timing and duration of hormonal therapy. The following relationships appear to be reasonably well established (Gaines *et al.*, 1938; Salmon, 1941). The monthly administration of less than 250 mg. of TP is usually unassociated with significant changes in the regularity or character of the menstrual flow, whereas 300 mg. of TP or more per month or its equivalent will usually modify menstrual bleeding. If treatment with these larger doses is initiated mid-menstrually, the appearance of the next menstrual flow is commonly accelerated, often by as much as 6–8 days. When hormonal treatment with these larger amounts is initiated at, or shortly after, the onset of menstruation and continued throughout the first half of the cycle, the next menstrual flow is generally delayed 1–4 weeks. After the administration of androgens in suppressive doses for 4 weeks or longer, menstruation may be resumed approximately 4 weeks after the last treatment, but may be occasionally deferred for several months. These variations in the time at which menstruation is reestablished appear to be related to the quality of ovarian function prior to treatment. The normal character of the reestablished menstrual cycle has been frequently demonstrated by the rapidity with which pregnancies have occurred in such subjects. In addition to irregularities in the intermenstrual interval, alterations in duration and amount of flow also occur. These changes are not uniform. The flow is usually reduced in amount, particularly in women with previous menorrhagia, but the duration may be either shortened or prolonged.

6. Breast

Observations on the effects of androgens on the normal human breast are limited to a few scattered and divergent reports as to gross changes in size during the prolonged administration of androgens. Atrophy was observed by Palmer and De Ronde (1943) after the administration of 650 mg. in 35 days; on the other hand, no perceptible changes in breast size were observed after the administration of massive doses of TP for many months to women with metastatic carcinoma (Herrmann, personal communication, 1946).

7. Virilizing Effects

Masculinizing tumors in women are accompanied by a variety of virilizing phenomena such as hypertrichosis, acne, enlargement of the clitoris and voice changes; all these have been described in women receiving androgens therapeutically. Their development and extent appear to be dependent not only upon the amount and duration of hormonal treatment but on individual susceptibility. There also appear to be different thresholds for the several virilizing phenomena observed.

a. Acne. The present viewpoint as to the relation of androgens to acne has been well presented by Hamilton (1941). At least two effects are believed to be exerted by androgens, an increase in sebaceous secretion and a hyperplasia of the epithelial cells at the mouth of the hair follicle. Both mechanisms favor comedo formation, upon which inflammatory and infectious processes are superimposed and lead to papule and pustule formation. The initial tendency for acne formation in puberal males tends to diminish with time, presumably through an adjustment between secretion and excretion of sebum. Acne is a common occurrence during the administration of androgens to women. The threshold for its development appears to be lower than for hypertrichosis, but there are pronounced individual differences. The acne induced has the distribution usual for spontaneous acne, that is, over the face, back and chest; and it likewise tends to diminish in severity with continued hormonal treatment (Abel, 1945). It appears to be an entirely reversible phenomenon except in those subjects in whom it occurs spontaneously.

b. Hypertrichosis. Hirsutism following the administration of androgens usually begins as a lanugo on the upper lip and later involves the chin, sides of the face and upper back; with prolongation of treatment, hair growth has been observed over the rest of the body. The hair may vary from a fine down to coarse long hairs. The hypertrichosis is particularly noticeable in brunettes and is generally more marked in normally hirsute subjects. As regards the influence of dosage, it has been stated that hirsutism rarely occurs unless 500 mg. of TP or more are given monthly (Salmon, 1941). Even in this dosage range, hypertrichosis occurred in only 1% of his series of 364 cases. However, variations in individual susceptibility are apparent from many reports of significant hirsutism from much smaller doses. Extreme hypertrichosis comparable to that seen in masculinizing tumors has been noted in women given massive and prolonged treatment with androgens for metastatic carcinoma (Adair and Herrmann, 1946). The suggestion that MT is less likely to induce hypertrichosis than an equivalent amount of TP (Salmon, 1941) warrants further study. The rate of axillary hair growth in hypogonadal women following the local or systemic administration of androgens has been suggested as a semiquantitative index of this action of androgens (Kinsell *et al.*, 1946). There is general agreement that the induced hypertrichosis undergoes a complete reversal on cessation of treatment although many months may be required for its complete regression.

c. Vulva and Clitoris. Zuckerman (1937) observed enlargement of the clitoris in monkeys receiving TP in amounts sufficient to inhibit follicular maturation and luteinization. Enlargement of the clitoris as well as striking vulval hyperemia and congestion were first observed by Shorr, Papanicolaou, and Stimmel (1938) in menopausal women receiving 25 mg. TP daily. Since then, all degrees of clitoral changes varying from congestion to hypertrophy have been noted in the majority of patients receiving more than 300 mg. of TP monthly (Foss, 1938; Salmon, 1941). With smaller doses, these changes occur much less frequently and may be limited to vulval hyperemia and to increased sensitivity of the clitoris. On cessation of treatment, the hyperemia rapidly disappears and a regression of the clitoral hypertrophy takes place at a rate which is usually proportional to the extent of the previous enlargement and may take many months. The significance of the vulval hyperemia and changes in the clitoris will be discussed later in relation to libido.

d. Voice. Voice changes experienced by women following the administration of androgens vary from slight huskiness or hoarseness to the development of a typical masculine-pitched voice. Subjects may complain of a sense of laryngeal constriction and of easy vocal fatigability; as with adolescent boys, the voice may "crack." Goldman and Salmon (1942) observed changes in the vocal cords of 23 of 400 women treated with androgens. Of these 23, 21 had received more than 500 mg. of TP monthly while 2 had been receiving 225–250 mg. In all 23, indirect laryngoscopy revealed changes in the vocal cords similar to those occurring in males at puberty. The early changes consisted in a generalized suffused swelling with hyperemia of the blood vessels running the length of the cords; the late changes were manifested by the assumption of a pale off-white color. In 12 cases, discontinuation of therapy was followed by the return of the voice to normal and a complete regression of the vocal cord changes. In the remainder, who were followed for as long as 2½ years, although the swelling and congestion usually disappeared, the vocal cords remained thicker and more fibrous than usual for women. In 3 subjects, the masculine quality of the voice persisted in spite of the complete regression of the cord changes. As regards the influence of dosage, experience suggests that individual differences in susceptibility exist so that no hard and fast rule can be laid down as to the innocuousness of any dosage range. Because of the potential irreversibility of the voice changes induced by androgens, they are the most significant of the masculinizing effects and frequently present the limiting factor in therapy.

Virilizing phenomena inherent in the therapeutic use of androgens constitute undesirable side effects. However, since they all appear to be entirely reversible, except for the voice changes in some instances, they should not be regarded as deterrents to the clinical use of androgens but rather as signposts for safeguarding therapy. Except for the voice changes these temporary aesthetic disadvantages must be weighed against the therapeutic benefit which may be anticipated. The virilizing effects may represent direct action of androgens on the structures influenced, or result from a disturbance of the usual estrogen-androgen balance in women by which the estrogens ordinarily preserve normal feminine characteristics.

8. Urinary Tract

a. Kidney. The effects of androgens in experimental animals in augmenting kidney weight and increasing its content of arginase and acid and alkaline phosphatase, have already been dealt with by Kochakian in

Vitamins & Hormones (1946). Sex differences in renal weights have also been observed in man, kidneys from males being significantly heavier than those from women (Lattimer, 1942). In the light of these findings the possibility has been explored that androgens may exert an effect upon kidney function. Careful studies of a variety of kidney functions during the administration of TP in amounts of 15-300 mg. daily have revealed no demonstrable alterations (Lattimer, 1942; Klopp *et al.*, 1945).

b. Bladder. Muellner and Hamilton (1944) in a well controlled study observed an increased tonus of the bladder following the administration of androgens to both men and women complaining of frequency and stress incontinence. After treatment, intravesicular pressures were higher and increased progressively with the introduction of larger amounts of fluid into the bladder. These effects were accompanied by an increase in force of the stream. Their results would suggest a direct effect of androgens in increasing the tonus of the bladder musculature. This effect is analogous to the well established action of androgens in inducing hypertrophy of skeletal musculature in experimental animals (Papanicoloau and Falk, 1938; and Kochakian, 1946). However, it should be pointed out that this does not necessarily imply a similar action of androgens on both types of muscles, in view of the many instances in which striated and smooth muscle respond differently to metabolic factors, as for example, in Graves' disease and progressive muscular dystrophy. There is one isolated finding which is not consistent with the observations of Muellner and Hamilton (1944). A cystometric study by Greenblatt (1942a) of one menopausal subject with frequency, urgency and incontinence showed an increased bladder capacity and a greater tolerance to bladder pressure following the implantation of TP pellets; a possible explanation for the reduction in bladder irritability might reside in the relief of emotional tension induced by androgens in this menopausal subject.

9. *Urinary 17-ketosteroids*

Increases in urinary 17-ketosteroids as measured by the Zimmerman reaction follow the administration of TP or T. The increase following TP is relatively small (Callow, Callow and Emmens, 1939) whereas a high percentage of injected T may be excreted (Dobriner and Shorr, unpublished data, 1946). On the other hand the administration of MT lowers the excretion of urinary 17-ketosteroids. An excellent comparison of the relative effects of TP and MT on 17-ketosteroid excretion, as well as a discussion of the mechanism responsible for the lowered excretion following MT, has been presented by Reifenstein *et al.* (1945).

10. Metabolic Effects

Within recent years, androgens have been found to exert a variety of metabolic effects which not only have great interest but important therapeutic potentialities. This aspect of androgen function has been the subject of an exhaustive review by Kochakian (1946) to which the reader is referred for details relating to nitrogen and phosphorus storage, energy metabolism, creatine-creatinine metabolism and the regulation of the storage and excretion of electrolytes such as sodium and potassium. Calcium metabolism has been reported to be affected in no regular fashion in normal subjects, but significant calcium retention has been observed in Cushing's syndrome (Albright, Parsons, and Bloomberg, 1941) and will be discussed more fully under this topic. The property inherent in androgens of building protoplasm has been the subject of increasing attention of late; and this group of hormones gives promise of becoming an important adjuvant in the management of many clinical conditions unrelated to disorders of sexual physiology.

11. Vascular Effects

As will be pointed out in the subsequent discussion on menstruation, there has been a growing appreciation of the major role played by vascular phenomena in the induction of menstrual bleeding. Almost nothing is known of the manner by which androgens may affect these vascular reactions in the uterus. Some observations have been made, chiefly on males, of their effects on the blood vessels of the skin. Edwards *et al.* (1939, 1941) using a Hardy spectrophotometer showed a diminished amount of hemoglobin, with a higher proportion in the reduced state, in the skin of castrate males as compared with normal controls. Within an hour of the administration of 20 mg. TP, there was an increase in hemoglobin concentration as well as in the percentage of oxyhemoglobin. Reynolds *et al.* (1942) studied the dermo-vascular actions of androgens in castrate, eunuchoid and normal males. As compared with normal controls, the hypogonadal males exhibited a greater irritability of the small cutaneous vessels to mechanical stimulation. The threshold for irritability was rapidly raised in normals and even more markedly in castrates by the injection of TP. Plethysmographic studies of the finger volume of castrate males showed a significant increase following TP, equivalent to that previously observed in normal males and menopausal women to follow the injection of estradiol benzoate. These vascular changes appeared within an hour of the injection, persisted at high levels for about 4 hours and returned to normal values by the 6th hour. These vascular phenomena have provided the stimulus for the use of androgens in peripheral vascular and coronary artery disease, a clinical application which has been discussed by Heller and Maddock in this volume.

12. Psychobiological Effects

The earliest use to which androgens were applied clinically was for the relief of the variety of vasomotor, psychological and emotional symptoms of the menopausal syndrome. The manner in which these phenomena are influenced remains entirely obscure.

IV. CLINICAL USE OF ANDROGENS

1. Uterine Bleeding

a. Functional. Functional bleeding may be defined as abnormal uterine bleeding occurring in the absence of organic disease. Uterine bleeding is regarded as abnormal when it exceeds the normal flow in duration, amount or frequency, or when it occurs intermenstrually. It is the prevailing opinion that these disturbances are largely of hormonal origin.

Pathophysiology. The point of view as to what constitutes menstruation has undergone considerable revision in recent years. The term was formerly reserved for spontaneous cyclical bleeding from a secretory endometrium (Schröder, 1928). This definition, which would restrict menstrual bleeding to ovulatory cycles, has been modified by two significant findings; the frequency with which regular bleeding has been found to be associated with anovulatory cycles in the monkey and man (Allen, 1927; Corner, 1927; Hartman, 1932, 1945; Novak, 1939) and the uniformity with which regular bleeding may be induced, in the absence of spontaneous ovarian activity, by the administration of estrogens alone (Allen, 1927). Although estrogen-induced bleeding usually follows the discontinuation of its administration, bleeding has also been observed to occur at fairly regular intervals during the continuous administration of estrogens to primates (Hartman and Firor, 1937; Zuckerman, 1941) and man (Reynolds *et al.*, 1940; di Paola and del Castillo, 1942). A second important advance in our understanding of the mechanisms of the menstrual process derives from recent studies on the morphological (Daron, 1936; Bartelmez, 1937; Okkels and Engle, 1938) and functional (Markee, 1940) characteristics of the vascular bed of the uterus. Their studies have shown that two types of arteries furnish blood to the endometrium; both branch off at right angles from the arteries in the subendometrial layer of the myometrium. The first type or straight artery supplies the basal portion of the endometrial glands and stroma and is independent of hormonal influences. The second type, the spiral artery, extends upward through the endometrium to within a short distance of the surface epithelium, and presents a typical corkscrew appearance, the number of whose coils varies with the stage of endometrial development. The growth, development and regression of the spiral arteries are conditioned by the action of the ovarian hormones. The study of the behavior of these vessels in ocular endometrial transplants in the monkey by Markee (1940) has disclosed a specific dynamic relation of the spiral artery to menstrual bleeding. The growth of the endometrial implant under the influence of endogenous or exogenous ovarian hormones is accompanied by a parallel but disproportionate growth of the spiral arteries which results in their progressive coiling. When the anabolic phase is interrupted by the cessation of secretory ovarian activity, or by hormonal withdrawal, characteristic changes take place in the behavior of the spiral arteries and in the endometrial transplants.

The initial change observed is a regression in the mass of the transplants through loss of interstitial fluid, which reaches considerable magnitude prior to the onset of bleeding. The reduction in the size of the implant leads to the compression and progressive coiling of the spiral arteries and to vascular stasis, usually accompanied by general vascular dilatation. About 4–24 hours before the first hemorrhage, vasodilatation is replaced by profound vasoconstriction of the spiral arteries, the site of the vasoconstriction being located in the deepest portions of the implant. There is marked blanching of the area supplied by the constricted artery, indicative of local anoxia, which is in turn responsible for the endometrial necrosis and its eventual sloughing. In one after another of the constricted arteries the blood flow is temporarily and briefly restored, and hemorrhage occurs through defects in the arteriolar wall and from capillaries and venules with or without the intermediary formation of hematomata. Cessation of bleeding takes place through final vasoconstriction of the arteriole with thrombus formation. Throughout this process there is progressive shedding of the compacta and spongiosa; thereafter rapid re-epithelialization takes place from the residual basal portions of the uterine glands, which have been maintained intact by a continuous circulation through the straight arteries. Regression of the endometrial transplant continues throughout menstruation and for a short period thereafter, and is terminated by the regrowth of the vascular bed.

Particular stress has been placed by Markee (1940) on the necessity of rapid regression as a preliminary to bleeding, which can be prevented by measures which retard the regression, as, for example, by a gradual reduction in hormonal dosage. He further emphasized the absence of fundamental differences between the endometrial and vascular changes in ovulatory and anovulatory cycles, or after hormonal administration, providing comparable endometrial growth has been achieved. Of particular relevance are his observations as to the influence of TP on the behavior of the endometrial transplant which is being maintained by daily injections of a constant dose of estrogen. Under these conditions TP induced a regression in the size of the implant during the period of its administration and for several days thereafter, comparable to that which results from a gradual reduction of administered estrogen. He was also able to prevent, by the administration of TP, the menstrual bleeding which ordinarily follows estrogen withdrawal. These results would indicate a direct inhibition by TP of the anabolic action of estrogens on endometrial growth.

It is still uncertain whether the factors responsible for these profound vascular changes which occur just preceding and during menstrual bleeding are of general or local origin, or both. The possibility that local toxic factors are involved as a result of the anoxic disintegration of the endometrium is now under investigation (Smith and Smith, 1946). These investigators have isolated from the uterine discharge a euglobulin similar to "necrosin" of Menkin (1943) which is toxic to rats and which they suggest may have local vascular effects of such a character as to serve as a precipitating factor initiating menstrual bleeding.

Much attention has been given to the histology of the endometrium in functional bleeding in order to determine whether the basis for the bleeding might exist in functional abnormalities in the structure of the endometrium. For many years emphasis was placed on the association of such bleeding with hyperplasia of the endometrium resulting from the persistence of Graafian follicles which undergo atresia rather than corpus luteum transformation. This view has been superseded in recent years by the recognition that, although the majority of cases are associated with endometrial hyperplasia, functional bleeding may occur from all types of endometrial patterns (Hoffman, 1944; Jones, 1938). The incidence of hyperplasia in functional bleeding varies from 23 to 68% in reported studies, suggesting a lack of uniformity in the standards for histological differentiation. Of particular interest are the mixed endometrial pictures designated by Traut and Kuder (1935) as irregular shedding or irregular ripening. In the former, the histological picture of the glands and stroma indicates an abnormal type of regression; in the latter an irregular development of the endometrium is evidenced by the presence of both secretory and proliferative types of endometrial glands. These abnormal endometrial patterns were found in one-third of their series of functional bleeders.

Even in the hyperplastic types, the underlying hormonal factors may not be uniform. Vaginal smear studies of functional bleeding have revealed two predominating types associated with hyperplasia of the endometrium (Shorr, 1945). In the more common type, a high degree of vaginal cornification persists, with little significant alteration throughout the menstrual cycle; the second type is characterized by the absence of cyclical changes and the persistence throughout the menstrual cycle of a relatively uniform vaginal smear characteristic of ovarian insufficiency. Both represent anovulatory cycles and both lead to the development of endometrial hyperplasia. The mechanism by which persistent estrogenic activity of a high degree leads to endometrial hyperplasia is readily apparent; the manner in which hyperplasia develops in chronic ovarian insufficiency is still uncertain. Markee's observations (1940) that low grade anovulatory cycles in the monkey may lead to regular bleeding but little or no shedding of the endometrium, may provide an explanation for this phenomenon. In the absence of adequate shedding, each succeeding menstrual cycle would have an additive effect on endometrial growth leading eventually to hyperplasia.

The variable endometrial picture in functional bleeding has led to the suggestion that the essential defect may lie in the myometrium. Two factors have been considered in relation to the possible effect of myometrial function on uterine bleeding. The first consists in the manner in which variations in spontaneous uterine contractility might affect blood flow to and from the uterus. The second relates to the possible influence on the endometrial blood flow of the arrangements of the myometrial fibers around the blood vessels, particularly in the inner quarter of the myometrium, as well as the presence of local aggregations of muscle fibers adjacent to the myometrial blood vessels, the "Polsters" of Burcura (1910). Both factors may alter the blood flow to the endometrium. As regards the influence of androgens on the myometrium, the meager information available at present has already been discussed and the suggestion that the effects of androgens on uterine bleeding is due to its influence on the myometrium (Abarbanel, 1940b) must be regarded as still inferential although not excluded. It is noteworthy, however, that the entire sequence of vascular events leading to menstruation were observed by Markee (1940) in endometrial transplants devoid of myometrium. This account of what is known of normal and abnormal uterine bleeding makes evident the complexity of these processes and the difficulty in analyzing the manner in which they may be influenced by any hormonal agent.

Therapy with Androgens. The first report of the use of androgens in functional bleeding was that of Loeser (1938a). In his series of 10 cases, including 5 suspected of having small intramural fibromas, TP in doses of 50 mg. were employed every other day for periods of 2 weeks or more with a total dosage of 300 mg. or more. A favorable effect on menorrhagia was uniformly observed. In those cases receiving a monthly total of 300 mg. TP, menses were delayed 8–10 days. In those receiving 500 mg. or more menses were suppressed and endometrial biopsies revealed a transition from a "normal" or hyperplastic endometrium to an atrophic one. Since this report, there has been an extensive literature dealing with the effects of androgens on functional bleeding and employing a wide variety of therapeutic regimes, from doses which suppress menstruation to those without observable effects on the menstrual cycle; with all, equally favorable therapeutic results have been reported.

High Dosage Regime. In the early studies it was more usual to employ androgens in amounts sufficient to suppress or significantly delay the subsequent menstrual flow and to produce regressive changes in the endometrium, as exemplified by the study of Loeser (1938a). Geist *et al.* (1938a) treated 25 cases of abnormal bleeding with 300–1000 mg. TP per month. Bleeding was controlled in 23 of 25 cases; endometrial biopsies showed varying degrees of regression, in some instances to atrophy. This series was subsequently enlarged (Salmon *et al.*, 1941a) to 45 cases. A large but unspecified number received 350–1000 mg. TP monthly with the induction of temporary amenorrhea. The initial results were uniformly good. The late results indicated recurrences in 6% and the persistence of only moderate improvement in 25% of this series. Mazer and Mazer (1940) administered 300–600 mg. TP per month to 20 patients with functional bleeding with effective control of bleeding during therapy and for 3 months after discontinuation, the period of observation. Similar results were reported by Foss (1938), Greenhill and Freed (1939), and Huffman (1940).

Medium Dosage Regime. It was soon appreciated that equally successful therapeutic results could be achieved with less than suppressive doses. Mazer and Mazer (1939), employing doses of 30–300 mg. TP per month, reported effective control of abnormal bleeding during and for at least 4 months after therapy in approximately 70% of 38 women. A comparison of these results with those they obtained with 300–600 mg. per month reveal an equal percentage of favorable results, averaging 66%. Geist and Salmon (1941) obtained equally good results from the use of 150–250 mg. TP monthly with those which they had previously reported to follow the use of suppressive doses of androgens. The immediate results were favorable in 76% of a series of 33 cases and were associated with the restoration of normal menses; moderate improvement occurred in 15% and no benefit in 9%. In those cases which had responded well, 60% remained free of excessive bleeding for periods of 6 months to 3½ years; 27% recurred but responded to supplementary androgenic therapy; in 13% the recurrence was complete. For such recurrences the authors suggested prolonged administration of 10–20 mg. TP weekly or its equivalent of MT. Other workers have preferred to limit the administration of these moderate amounts of androgens in functional bleeding to the first or second half of the menstrual cycle and have reported equal benefit with these regimes (Coggi, 1939; Greenhill and Freed, 1939; Black, 1942; Jacoby and Rabbiner, 1943; Simon, 1946). The experience of Greenblatt (1943a) with the implantation of pellets of TP should also be considered as a medium dosage regime. The pellets varied in weight from approximately 100–400 mg. and were implanted in 8 patients with severe functional bleeding who had failed to respond to previous curettage and a variety of other therapeutic procedures. In 5, the bleeding was associated with cystic glandular hyperplasia or persistent estrogenic endometria. In all, menstrual bleeding was reduced to normal. In 3, despite the control of menorrhagia, endometrial biopsies revealed persistent hyperplasia or estrogenic endometria for many months after pellet implantation. Stabilization of the menses was stated to be the rule although a temporary cessation or an increase of the intermenstrual interval frequently occurred.

Small Dosage Regime. A number of studies have utilized much smaller amounts of androgens, ranging from 30–100 mg. of TP or its equivalent per month. The most extensive studies of the effects of small amounts of androgens on functional bleeding are those of Sturgis, Abarbanel *et al.* (1940) and Abarbanel (1940b). Fourteen cases with various endometrial patterns were given 5–25 mg. TP at 2–4 day intervals for 2–4 doses. The usual amount required to lessen bleeding was stated to be 10–30 mg.; to stop the flow 10–120 mg., usually 40–60 mg. A sharp decrease in flow was generally observed within 48 hours of the initial injection. In 3 refractory patients TP pellets totalling 24–28 mg. proved effective. Other studies using small doses of TP include the following: Béclère (1938, 1939a,b; 1941, 1945?); Rubenstein (1939); Bishop (1939); del Castillo and Salaber (1939); Desmarest and Capitain (1939); Huffman (1940); Greenblatt (1940, 1943b); Mandy and Mandy (1940); Leff (1941); and Beato (1946).

Prophylactic Use of Androgens. Once the specific bleeding episode has been controlled, the subsequent use of androgens is dependent upon whether bleeding recurs. As with all methods which have been successful in controlling acute bleeding episodes and which include curettage, estrogens and progesterone, a considerable number of patients will remain free of bleeding for varying lengths of time; in others, bleeding will recur and require prolonged therapy. In such cases androgens have been effectively employed by a variety of regimes. In general, the amounts found effective for this purpose are smaller than those required to interrupt a bleeding episode. Successful management has been reported to be achieved by the administration of androgens in doses of 5–10 mg. MT daily, or 10–20 mg. TP weekly throughout the cycle (Geist and Salmon, 1941; Leff, 1941) or by the implantation of pellets (Greenblatt, 1943a). Other variations, such as the selection of the first or second half of the cycle for therapy, appear to be equally effective (Desmarest and Capitain, 1939; Greenhill and Freed, 1939; Sturgis *et al.*, 1940; Black, 1942; Jacoby and Rabbiner, 1943).

Discussion. There appears to be general agreement that functional bleeding can be effectively managed in a large percentage of cases by means of androgens, but no certainty as to the manner in which the therapeutic effects are achieved. When suppressive doses are employed, the action of androgens may be considered equivalent to a "medical curettage" achieved by the inhibition of ovarian hormonal activity *via* the pituitary, although the neutralization of the effect of estrogens on the endometrium may also be a contributory factor. This explanation, however, does not apply to the apparent equal effectiveness of moderate and small dosage regimes which are unassociated with detectable influences on endometrial histology. The possible myometrial effects of androgens in influencing uterine bleeding remain, for the present, a matter of inference as far as man is concerned, but cannot be excluded. In view of the variety of endometrial patterns associated with functional bleeding and the importance of vascular phenomena for the menstrual process, it is not unlikely that the effects of the smaller doses of androgens on functional bleeding may be mediated through their influence on the dynamics of the endometrial vascular bed, a circumstance that would elude histological examination.

When relief from excessive bleeding persists after discontinuation of androgenic therapy, there is no present reason to attribute it to any corrective influence of androgens on the underlying defect in pituitary-ovarian function. It is more likely that any enduring effects are due to spontaneous corrective changes which follow curettage with equal frequency. When bleeding is prone to recur, the chronic use of small doses of androgens appears to be of prophylactic value.

The eventual place which androgens will assume in the management of functional bleeding will depend upon its merits relative to other measures such as the use of estrogens and, particularly, progesterone and on the future development of corrective rather than ameliorative measures.

b. In Association with Myoma. Androgens have also been used for the management of excessive uterine bleeding associated with the presence of myoma.

Pathophysiology. Myomas have their highest incidence between the ages of 30 and 45 and are relatively infrequent prior to the fourth decade (Novak, 1941). Their presence may be compatible with normal menses or accompanied by all degrees of abnormal bleeding. The latter condition is usually associated with the submucous or intramural location of the myoma. The bleeding may arise from erosions of the overlying endometrium, from interference with the normal uterine vascular pattern, or from a concomitant hyperplasia of the endometrium. The frequent coexistence of myoma with endometrial hyperplasia has led to the suggestion that both conditions may arise from a common hormonal stimulus and that the abnormal bleeding under these circumstances may be due to the endometrial hyperplasia *per se.* An hormonal relation to myomas is suggested by their frequent reduction in size after the menopause or following surgical or X-ray castration as well as by their experimental production in the guinea pig by estrogens (Lipschütz *et al.*, 1939). Of particular relevance to the therapeutic use of androgens is the demonstration by the same investigator that myoma production by estrogens is prevented by the simultaneous administration of androgens in a ratio of 1:22.

Therapy with Androgens. The clinical use of androgens for the management of abnormal bleeding associated with myomas has been much less extensive than for uncomplicated functional bleeding and the results less satisfactory. Loeser (1938a) treated five cases in which uterine bleeding was complicated by the presence of small intramural fibromas, with 50 mg. TP every other day for two weeks or longer with the control of the excessive bleeding and a perceptible reduction in the size of the myomas. The most extensive report is that of Geist and Salmon (1941) who treated 21 cases of uterine bleeding associated with fibroids, employing high and medium doses. The initial results were quite favorable, with good results in 71%, moderate improvement in 19% and no benefit in 10%. A follow-up of 19 of these cases for periods of from 3 months to 4 years showed that only 37% had a restoration of normal menses and 42% a recurrence of abnormal bleeding. There are numerous other reports comprising small series or single cases with essentially the same findings (Béclère, 1938, 1939b,c, 1945?; Palmer and Moricard, 1938; Turpault, 1939; Fabre, 1939; Greenhill and Freed, 1939; Loeser, 1940; Huffman, 1940; Greenblatt and Wilcox, 1941; Salmon *et al.*, 1941a; Perloff, 1942; Black, 1942; Douay, 1945?). Greenblatt (1943a) treated 12 cases of severe uterine bleeding in association with myoma by implantation of TP pellets, totaling 25–300 mg. Twenty-five per cent experienced no relief; in 75% bleeding was alleviated, in some instances for as long as 18–22 months. In spite of the temporary benefit achieved, this author makes it clear that he regards this measure as palliative rather than remedial, and not a substitute for surgery.

Discussion. The mechanisms by which androgens control bleeding associated with myoma are probably similar to those discussed under functional bleeding. In general, the therapeutic results have been less satisfactory than in the treatment of uncomplicated functional bleeding and frequently are of a temporary character. No regular effect has been described on the size of the myoma. This form of therapy should not be considered an effective substitute for more radical procedures.

2. Pelvic Disorders

a. Endometriosis. Endometriosis is a condition in which tissue resembling endometrium occurs aberrantly, chiefly in the pelvic cavity.

Pathophysiology. The most common sites for endometrial implants are the ovaries, uterine ligaments, cul-de-sac and the pelvic peritoneum covering the uterus, tubes, rectum, sigmoid and bladder. The aberrant tissue not only resembles the uterine endometrium histologically but also responds to hormonal stimulation in a like manner. There is still much uncertainty as to the genesis of these implants. Of the several current theories, *i.e.*, tubal regurgitation of endometrial tissue (Sampson, 1927), lymphatic dissemination of endometrial tissue, and coelomic metaplasia (Novak, 1926; Meyer, 1930), the last is favored, although tubal regurgitation is regarded as an occasional mechanism. While endometriosis may occur at any period in the menstrual life, it is most common between the ages of 30 and 50. Although asymptomatic in a large proportion of cases, it is frequently associated with menstrual disturbances, with infertility (approximately 75%) (Sutton, 1940) and dysmenorrhea (38% of a series studied by Keene and Kimbrough, 1930). The dysmenorrhea may be of all intensities and pain is commonly referred to the rectum and lower back. Dyspareunia and painful defecation are not uncommon. The pain is believed to be due to an increase in size of the implants at menstruation, as well as to the pressure of retained menstrual blood in the cyst cavities. There is an occasional rupture with peritoneal irritation. The masses are tender and painful to palpation. Diagnosis is occasionally difficult and may rest on history and the presence of painful nodules, tenderness and thickening of the uterosacral ligaments. The customary treatment is surgical removal of the implants where possible, and, in more severe cases, more drastic measures such as surgical or X-ray castration for the purpose of removing the source of hormonal stimulation necessary for the survival of the implant.

Therapy with Androgens. The effect of androgens in suppressing menstruation and inducing regressive changes in the uterine endometrium provided the rationale for their trial in endometriosis, particularly in the child-bearing age, as an alternative to more drastic procedures. The first report was that of Wilson (1940) who administered a total of 4800 mg. TP over a period of 13 months, with the resultant suppression of menses, relief of pain and diminution in size of the pelvic implants. Virilizing effects including hoarseness, hypertrichosis and enlargement of the clitoris developed during therapy but regressed thereafter. Endometrial biopsies revealed atrophy throughout treatment. Following cessation of therapy, because of progressive masculinizing effects, ovarian function was promptly restored with a return of the pelvic tumor and dysmenorrhea. Relief of dysmenorrhea during the administration of 300–750 mg. MT per month, with subsequent return of pain on discontinuation of therapy, was reported by Salaber and del Castillo (1942) in 2 cases. These doses did not suppress menstruation. The following year, Salaber *et al.* (1943) confirmed the immediate effect of androgens in relieving pain in endometriosis. Symptomatic relief in 2 subjects during medication with 125–400 mg. TP per month and reduction of the endometrial mass, were reported by Hirst (1943). Williams (1943) and Miller (1944) reported the reduction of the endometrial lesions during the preoperative use of androgens and the latter also observed histological evidence of fibrosis. Berlind (1941) and Salmon (1941) comment on the probable usefulness of this method of therapy.

Our own experience (1941–1946) with this form of therapy has been derived from the study of 10 cases. The most usual regime employed was the administration of 25 mg. TP 6 times weekly for 4 weeks, starting mid-menstrually. Five cases received one such course of therapy, which usually leads to the suppression of one menstrual cycle. Three have had complete relief for 3, 3 and 4 years; one a recurrence after 4 years with complete relief following a second course of treatment 1 year ago; and 1, a recurrence after 2 years which was terminated by operative removal of a chocolate cyst. Of the remaining 5, 1 required 2 courses of treatment with relief persisting for 2½ years to date and 1 with massive endometriosis required treatment over a period of 16 months, during 11 of which she received therapy. Treatment was interrupted twice with return of severe symptoms after 2–3 months. Initially, suppressive doses of 500 mg. TP were employed; thereafter non-suppressive doses of 130–160 mg. TP per month were given with progressive reduction and eventual abolition of all discomfort. Complete relief has now persisted for 2½ years without further therapy. Masculinizing phenomena were prominent in this case, consisting of hypertrichosis, enlargement of the clitoris, increased libido and acne, but voice changes were absent; all virilizing changes have completely regressed. In the other patients, virilizing phenomena were minimal with transient hoarseness in two. Two patients are still under treatment; and there has been one complete failure. These extremely favorable results in this small series may not be representative, as is evident from other reports cited, but are indicative of the potential value of this therapeutic regime.

Discussion. As an alternative to the more radical procedures which might otherwise be required for the relief of endometriosis associated with dysmenorrhea in the younger age group, the use of androgens is rational and the results sufficiently promising to warrant their trial before resorting to more drastic procedures. Not a few cases may be salvaged by adequate androgenic therapy, although a proportion of cases may prove to be refractory. Further experience is required before the optimal regime and mode of administration can be determined. The relative merits of suppressive *versus* non-suppressive dosage regimes, including pellet implantation, are now under investigation.

It is suggested that differences in the character of the vascular supply may be responsible for the permanent atrophy of aberrant endometrial tissue as contrasted with the temporary regression of the uterine endometrium. The two-fold character of the arterial supply to the uterine endometrium insures the survival of the basal portion of the endometrial glands, which receive a continuous supply of blood *via* the straight arteries independently of hormonal influences and from which the endometrium is subsequently reconstituted. The aberrant endometrium is not known to possess a similar vascular organization so that, when regression occurs, it is complete and irreversible. The occasional recrudescence, 3–4 years after an induced remission, may not be due to the persistence of the original endometriosis but to the development of new implants.

b. Pelvic Inflammatory Disease. Pelvic inflammatory disease is prone to premenstrual and menstrual exacerbations and is frequently associated with dysmenorrhea. With a view to avoiding these exacerbations, Stone (1941) treated 6 cases of pelvic inflammatory disease with 350–650 mg. TP per month, which was sufficient to suppress menstruation. Improvement of the pelvic inflammatory disease was observed in 5 of the patients; in 4 there was a reduction in the size of the pelvic mass; in 1, a complete disappearance. In a report by Turpault (1945?) which is difficult to evaluate, amelioration of menstrual exacerbations were reported to follow the injection of 10 mg. TP directly into the cervix on the 5th and 3rd days prior to menstruation. The absence of further reports on the use of androgens for this condition is very likely attributable to the effectiveness of the newer chemotherapeutic agents in the management of this disorder, particularly during the acute stage. However, androgens may still prove to be useful adjuvant therapy for the dysmenorrhea associated with the chronic types of pelvic inflammatory disease.

c. Endocervicitis. The use of androgens for this condition is limited to a single case to which 750 mg. TP was administered over a 6 week period (Wollner, 1941). Cervical biopsies revealed a decrease in the number of glands with a change from a high to a low columnar nonsecreting type of cell, a decrease in cervical secretion and the transformation of a hyperemic and edematous stroma to a dense, anemic one. One area showed metaplastic changes in the squamous epithelium.

d. Malignancy of the Genital Tract. In view of the increasing interest in the possible therapeutic value of androgens in malignancy, the reports of their effects on pelvic malignancy warrant discussion in as much detail as the available reports permit. Beecham (1943) treated 6 cases, 3 with ovarian carcinoma, 1 with carcinoma of both ovary and breast and 2 with carcinoma of the cervix. The total dosage varied from 270 to 1310 mg. TP given in doses of from 10 mg. thrice weekly to 25 mg. daily. Five of the 6 patients experienced temporary subjective relief with abolition of pain, weight gain and increase in well-being. There was no evidence that the course of the malignancy was altered, as judged from the subsequent course of the patient and the absence of demonstrable histological alterations in the 2 cases in which such studies were made. Two patients with ovarian carcinoma were given TP by Wyatt (1945); 1 received 7200 mg. TP over a 5 month period; the other 3150 mg. TP over 3 months. Both experienced temporary improvement in well-being and then became progressively worse and died. There was no change in the histology of the tumor in either case. Abel (1945) employed TP or MT in 5 patients, 3 with carcinoma of the cervix, 2 with carcinoma of the body of the uterus. Each patient received either 600 mg. TP or MT per month for approximately 10 months. All experienced temporary increases in well-being and a gain in weight. The evaluation of the effect on the pelvic neoplasms was limited to recto-vaginal examinations, and the eventual outcome unreported. There were no detectable alterations in the pelvic masses during therapy. It may be concluded from these reports that, although an increase in weight and well-being and a reduction of local discomfort were achieved by androgens, the course of uterine, ovarian and cervical carcinoma was unaltered.

e. Functional Urinary Disturbances. Several studies have been made of the effect of androgens on urinary bladder symptoms such as urgency, frequency, dysuria, incontinence and diurnal and nocturnal frequency, presumed to be of functional origin. In many of the patients these symptoms were associated with the menopausal syndrome; in others uterine fibroids were present. Mocquot and Moricard (1936b) reported relief of such disturbances in patients with menopausal symptoms, using 10–20 mg. TAc per month for 4–5 months. These very small doses were said to be adequate to relieve both the nocturia and the menopausal symptoms. Beckers (1945) reported relief of nocturia and menopausal symptoms following the administration of 25 mg. TP weekly for 6 weeks, or the implantation of 75 mg. pellets of T. These results are in contrast with the experience of Geist and Salmon (1943) who treated 9 postmenopausal patients complaining of dysuria, frequency or incontinence with 25 mg. TP thrice weekly for 4 weeks; only 2 were slightly improved; 5 showed no improvement, and the symptoms were aggravated in the remainder. Most of these cases had previously obtained symptomatic relief with estrogens. The reasons for these discrepancies may reside in the considerably larger amounts of TP employed by Geist and Salmon (1943) which may have induced regressive vaginal changes and an irritative vaginal discharge.

In the premenopausal age period, a high incidence of similar disturbances of micturition, particularly nocturnal frequency, was observed by Greenblatt and Wilcox (1941) and Greenblatt (1942a,b) in patients with massive uterine fibroids, as well as in association with a variety of menstrual disturbances. Relief regularly followed the implantation of TP pellets weighing 25–400 mg., with an average dosage of 100 mg. This relief was accompanied by no significant reduction in the size of the myoma. Greenblatt concluded that the influence on micturition was a direct result of the action of androgens on the bladder musculature, the evidence for which has been discussed above.

3. Dysmenorrhea

Primary (functional, essential) dysmenorrhea is the term applied to painful menstruation in the absence of demonstrable organic pelvic disease.

Pathophysiology. It is the prevailing opinion that dysmenorrhea characteristically occurs in essentially normal ovulatory cycles (Novak and Reynolds, 1932; Wilson and Kurzrok, 1938; Sturgis and Albright, 1940); that this is not invariable, however, is evident from the studies of Lackner *et al.* (1937), who observed typical dysmenorrhea in women with proliferative, hypoplastic or atrophic endometrial patterns on the first day of menstruation. Because of its usual association with normal ovulatory cycles, the possibility has been considered that dysmenorrhea may result from abnormal uterine contractions which are dependent on previous priming with estrogens and progesterone. Studies of uterine motility in normal women have demonstrated characteristic contraction patterns for the different phases of the menstrual cycle (Wilson and Kurzrok, 1938; Bickers, 1941, 1942). The contraction waves during the follicular phase were frequent and of low amplitude; during the progestational phase there was a decrease in frequency, but an increase in height and duration, a pattern that was carried over into the early part of menstruation. Information is meager as to the specific action of hormones on uterine motility. In a case of primary amenorrhea rhythmic contractions were absent; combined treatment with estrogens and progesterone induced a typical progestational pattern which was duplicated by the administration of TP alone (Wilson and Kurzrok, 1940a). In one dysmenorrheic subject, 100 mg. TP given during the second half of the cycle was without influence on symptoms or uterine contractions (Bickers, 1941). Comparisons have been made of uterine motility during the menstrual phase of painless and dysmenorrheic cycles with discrepant results. Wilson and Kurzrok (1938, 1940b) observed no differences. On the other hand, Moir (1934) observed, in a single dysmenorrheic subject, a marked increase over the normal amplitude of contraction and a transmitted intrauterine pressure estimated at 30 mm. Hg above the systolic blood pressure at the height of contraction. He attributed the pain of dysmenorrhea to myometrial ischemia resulting from these pressure differences at the height of the uterine contraction. Bickers (1941, 1942) confirmed the similarity of the contraction waves during painless and painful menses but observed a relaxation to a lower base line after morphine in dysmenorrhea which was absent in the normal subjects. This difference was attributed to a sustained uterine "tetany" in dysmenorrhea on which contractions of normal character were superimposed, a situation consistent with the intermittent uterine ischemia theory of Moir (1934). More recent studies of Woodbury and his associates (Woodbury *et al.*, 1946; Woodbury *et al.*, 1947; Child *et al.*, 1946; Huggins and Woodbury, 1946) using multiple small balloons and more sensitive recording methods have revealed in dysmenorrheic women a variety of abnormal menstrual uterine contraction patterns, such as organized contractions which develop abnormally high pressures, disorganized contractions of low or high pressures, often of multiple origin, with contractions superimposed upon each other. In dysmenorrhea, the intrauterine pressures were usually significantly elevated above normal and frequently above the arterial pressure, a circumstance which would correspondingly interfere with uterine blood flow. These exaggerated contractions were occasionally restricted to one segment of the uterus and might not have been detected by the conventional single balloon technic. In control subjects, an elevation of intrauterine pressure sufficient to raise the contraction amplitude to levels characteristic of dysmenorrhea induced typical pain. Dysmenorrheic patients were extremely sensitive to uterine distention as well as to pitressin. The abnormal contractions in dysmenorrhea were attributed to an increased uterine irritability, not only to the intrauterine pressure due to accumulation of menstrual debris, but also to pitressin-like and other hormonal factors involved in the menstrual cycle. Agents which were effective in relieving pain reduced uterine irritability and, in some instances, converted disorganized into organized uterine contractions; although, in some subjects, relief of pain occurred with no marked alteration of the contraction pattern. These important studies, however, provided no information as to the factors responsible for the increased uterine irritability in dysmenorrhea of which the abnormal uterine contractility is the result.

A wide variety of therapeutic measures have been used in the management of dysmenorrhea. On the concept that dysmenorrhea is generally associated with normal ovulatory cycles, it has seemed logical to utilize measures which might interfere with the normal sequence of ovarian changes. The administration of estrogens, if instituted in the postmenstrual phase, prevents the development of a corpus luteum and inhibits progesterone formation, as is evident from the persistence of a proliferative endometrium. The relief which follows this regime (Wilson and Kurzrok, 1938; Sturgis and Albright, 1940) has been attributed to the elimination of the effects of progesterone on uterine contractions during the subsequent menses. On the other hand, favorable results in many cases of dysmenorrhea are reported to follow the premenstrual administration of progesterone (Lackner *et al.*, 1937).

Finally, it should be pointed out that there is a growing tendency to regard dysmenorrhea as a psychosomatic problem and to emphasize the importance of psychotherapy in its management. This concept is not incompatible with the observed abnormalities in uterine behavior but would regard the heightened uterine irritability as secondary to psychic and emotional stress. With this background, we may turn to the consideration of the place of androgens in the management of this problem.

Therapy with Androgens. The possible usefulness of androgens in the treatment of dysmenorrhea was first pointed out by Loeser (1938a) on the basis of favorable results in 2 cases. Since then, androgens have been extensively employed for this purpose in a variety of dosages and therapeutic regimes. These may be conveniently divided into three general categories on the basis of total dosage per menstrual cycle:

High Dosage Regime (300–900 mg. of TP per cycle). These amounts are sufficient to interfere with the normal develo ment of the ovarian cycle. Endometrial biopsies generally reveal an absence of the secretory phase, indicating the suppression of corpus luteum formation and progesterone production. In this respect this regime is analogous to the procedure which utilizes estrogen for the same purpose (*vide supra*). Employing these amounts of androgens, Salmon *et al.* (1939) reported complete relief in 73%, and partial in 15%, of a series of 30 patients. Almost 50% of the patients remained asymptomatic for 3–24 months after treatment was discontinued. Doses of the same order of magnitude were used by Greenhill and Freed (1939, 1940) with results which were somewhat less striking and uniform. In their series the hormone was given either during the first or last half, or throughout the entire cycle. Although the symptomatic results were extremely satisfactory, the masculinizing effects which usually resulted from this dosage range led to the exploration of the possibility that benefit might follow the use of smaller amounts of hormone which would be devoid of undesirable side effects.

Medium Dosage Regime (125–250 mg. of TP or its equivalent per cycle). These amounts of androgens are reported as inducing neither undesirable virilizing phenomena, significant menstrual irregularities, or discernible changes in the vaginal and endometrial pattern. Symptomatic relief comparable to that obtained with the higher doses was reported by Geist and Salmon (1941); 72% were completely relieved of symptoms and 16% considerably improved in a series of 26 patients. Six of 8 patients who were not given supplementary androgen therapy remained symptom free for from 4–16 months. The studies of Greenblatt (1943a) in which pellets of TP weighing between 150 and 400 mg. were employed may properly be included in this category. In a group of 9 patients studied for a total of 40 cycles for the entire group, 75% relief or better was reported for one-half, and appreciable relief during one-quarter, of the cycles.

Low Dosage Regime (125 mg. or less of TP or its equivalent, per cycle). The multiplicity of regimes and variations in dosage employed in this group of studies complicates their evaluation, but the clinical results were reported to be equally as good as with medium or large doses. Cinberg (1942) administered 10 mg. TP subcutaneously for 3 consecutive days prior to the expected flow, or 20 mg. MT for 10 days premenstrually, with complete relief in 64% and partial relief in 25% of 22 women; symptoms returned in all but two after therapy was discontinued. Excellent results were reported (Feiman, 1941) in 70% of cases given TP in amounts of 10 mg. on the fifth and 25 mg. on the second day preceding the flow. Ten mg. of TP given on alternate days, or 30 mg. MT given daily, for 2 weeks preceding menstruation, gave complete or partial relief in 67% of 18 patients; in 7 of 12 patients there was an abrupt return of symptoms on discontinuation of therapy (Jacoby and Rabbiner, 1943). Very successful results with even smaller doses were reported by Rubenstein and Abarbanel (1939), who found the subcutaneous administration of 5 mg. TP in oil 2–3 times during the month highly effective. In a few instances a total monthly dosage of 45 mg. was found necessary. There was complete relief in 16 out of 26 patients and partial in 4. An even more striking experience with the effectiveness of very small doses was reported by Abarbanel (1940a) using TP percutaneously in an ointment base or sesame oil. A total of 20–90 mg. of TP was employed locally in divided doses for 10–14 days premenstrually. In some instances as little as 5 mg. applied locally in sesame oil on the eighth and third day before the flow sufficed. The relative effectiveness of percutaneous TP was estimated to be $\frac{1}{3}$ to $\frac{1}{6}$ that by the subcutaneous route. On this basis there was a maximal total absorption of from 4 to 30 mg. of TP over a period of 10 days in this series. Nine of 10 cases reported complete relief, frequently of several months' duration following discontinuation of therapy.

Discussion. Uterine contractions during menstruation appear to be under the control of a constellation of ovarian and posterior pituitary hormones and influenced by the degree of intrauterine distention. From present evidence, dysmenorrhea is associated with abnormal uterine contractions which are attributed to an enhanced uterine irritability. The factors responsible for the increased irritability are still obscure. All published reports are in agreement as to the beneficial results of androgens in dysmenorrhea. However, equally favorable results were reported with a variety of dosage schedules and were apparently independent of the phase of the menstrual cycle during which the hormone was administered, although the smaller doses were usually given premenstrually.

With the higher dosage schedules, the association of symptomatic relief with the suppression of corpus luteum formation and progesterone production is analogous to the beneficial effects of estrogens given in amounts sufficient to induce anovulatory cycles. The relief in both instances is compatible with the concept that the removal of some component, presumably progesterone, from the hormonal environment reduces uterine irritability sufficiently to abolish abnormal uterine contractions. However, this explanation would not apply to the equally favorable results obtained with smaller, non-virilizing amounts of androgens, which induce no detectible changes in ovarian hormonal function. Relief of premenstrual tension, or subtle hormonal changes not discernible by present indices are not excluded as possible mechanisms, although in many instances benefit was reported with amounts of androgens much too small to be related to any known action in man. Under these circumstances, the attribution of specificity to the action of small amounts of androgens in as complicated a syndrome as dysmenorrhea seems hazardous, particularly when relief persists for many months beyond the actual period of therapy.

The success of all varieties of therapeutic measures, frequently with opposite physiological actions, suggests that psychological and emotional factors are prominent in the development and amelioration of this syndrome. This concept does not exclude a functional derangement in uterine contractility as the basis for the pain experienced, but seeks to provide a basis, on the behavioral level, for the increased uterine irritability responsible for the abnormal contractions.

4. Premenstrual Tension

Premenstrual tension is the term usually applied to a variety of symptoms such as nervousness, irritability, emotional instability, depression, headache, nausea and tension which occur premenstrually and which usually subside with or shortly after, the onset of menstruation. These symptoms may be of any intensity and vary in duration from one or two days to as long as the entire second half of the menstrual cycle. This term is occasionally used to include premenstrual "bloating" and edema.

Pathophysiology. There is no specific knowledge as to the mechanisms by which these psychic and emotional changes are related to the hormones involved in the ovarian cycle. Frank (1931) suggested that they might arise from an exaggerated premenstrual increase in blood estrogens which he had observed in women with premenstrual tension, as compared with normal subjects. Israel (1938) suggested that the premenstrual hyperestrogenemia reported by Frank might produce these symptomatic effects because of concomitant progesterone deficiency which permitted the estrogens to act in unopposed fashion. The effects of steroidal hormones on electrolyte and water metabolism has also led to the suggestion that premenstrual discomfort might arise from the influence of abnormal hormonal patterns on the retention of electrolytes and water in the extracellular spaces (Greenhill and Freed, 1941; Freed, 1945). These several theories have served as a basis for a variety of therapeutic regimes, such as the premenstrual administration of progesterone and diuretics such as ammonium chloride, and have led to the prevailing opinion that estrogens are contraindicated.

The concept of premenstrual hyperestrogenemia was, however, based on results obtained with a method for estrogen assay which did not detect the very high proportion of bound estrogens in the blood. A subsequent study of blood and urinary estrogens throughout the menstrual cycle by Markee and Berg (1944), utilizing more adequate methods, have failed to show any premenstrual rise in blood estrogens; on the contrary, they fell to lower levels and paralleled the lower urinary levels characteristic of the premenstrual phase. Our own concept of premenstrual tension would place it in the same category as the menopausal syndrome not only because of the similarity in symptomatology but also for its association with the waning phase of estrogen production during the menstrual cycle. We would accordingly consider the same predisposing factors as operative, namely, a psychological and emotional maladjustment to the temporary premenstrual reduction of the concentration of estrogen in the organism. In this clinic the use of supportive estrogenic hormone therapy has been adopted for the management of premenstrual tension with results comparable to those obtained in the menopausal syndrome (Shorr, unpublished data).

Therapy with Androgens. Androgens may now be added to the list of agents which have been found therapeutically effective in this syndrome. As with dysmenorrhea, equally successful results have been reported with a variety of dosage regimes ranging from the percutaneous application of as little as 2.5 mg. TP in sesame oil for 10 days premenstrually (Abarbanel, 1940a), to as much as 200 mg. TP given in divided doses during the second half of the cycle (Geist, 1941). It has been more usual to administer intermediate amounts, totaling 5–50 mg. TP in single or divided doses during the latter part of the cycle (Greenblatt, 1940; Geist and Salmon, 1941), MT, 10 mg. daily for 10–7 days before the expected flow (Freed, 1945) or TP pellets (Greenblatt, 1943a). Freed (1946) more recently has been employing T in aqueous suspensions, giving one injection of 20 mg. on the seventh day premenstrually. In addition to a striking immediate symptomatic effect, relief was reported to persist for many months after discontinuation of therapy.

Discussion. The manner in which the steroidal hormones are related to the genesis and relief of premenstrual tension is obscure. The specificity of the action of androgens or any other agent is difficult to evaluate in a syndrome which appears to be so profoundly influenced by personality factors and stress situations as well as doctor-patient relationships. This is evident from the relief reported in premenstrual tension from the use of a variety of therapeutic agents often possessing opposite physiological actions. It seems unlikely that the amounts of androgens found effective are adequate to influence significantly water and electrolyte metabolism; in any event their effects would be to favor further retention. The more likely explanation would be that androgens in moderate doses exert their effect in a manner comparable to their relief of the similar symptomatology of the menopausal syndrome, leaving the question of mechanism for elucidation. This suggestion is supported by the equal effectiveness of estrogens given during the second half of the cycle in ameliorating premenstrual tension. Some doubt may be expressed as to the specificity of the relief reported with the very small amounts of androgens used by some investigators, particularly when symptomatic relief persists for many months after therapy is discontinued. The usefulness of androgens as well as estrogens in premenstrual tension should not lead to the reliance on these agents to the exclusion of the more fundamental psychotherapeutic approach.

5. The Menopausal Syndrome

The earliest clinical use of androgens was their application to the management of the menopausal syndrome.

Pathophysiology. The menopause, whether due to the spontaneous aging of the ovary or induced by surgery or irradiation is accompanied by at least two characteristic alterations in the hormonal environment, a profound reduction in estrogen production and a heightened excretion of urinary FSH. To this altered hormonal environment, a considerable percentage of women react by the development of a wide variety of symptoms which include vasomotor reactions, psychic and emotional instability and other functional disturbances; whereas the remainder make an uneventful or relatively asymptomatic adjustment to this episode (Shorr, 1940b; Henry and Shorr, 1941). No measurable difference in the known hormonal changes of the menopause exists between these two groups. The opinion is widely held and would appear to be justified, that the psychological and emotional status prior to the menopause is a major determinant in the subsequent adjustment to the new hormonal environment. The therapeutic effect of estrogens in ameliorating the menopausal syndrome is well established but some difference of opinion still exists as to the mechanism by which relief is achieved; whether by the restoration of a more adequate concentration of estrogens in the organism or the reduction to normal levels of the heightened FSH excretion. The weight of evidence favors the former concept. Symptomatic relief with estrogens may be experienced with little or no reduction in urinary FSH (Heller *et al.*, 1944; Shorr, unpublished data). Furthermore, typical menopausal symptoms occur with non-endocrine pituitary tumors in association with subnormal urinary gonadotropin values (Shorr, *et al.*, unpublished data) as well as in young menopausal women in their twenties, in which the amenorrhea is due to a depression of pituitary gonadotropic function (Shorr and Carter, unpublished data).

The evaluation of therapeutic agents may be complicated by several circumstances. Occasional brief periods of spontaneous ovarian activity may occur which are associated with heightened excretion of estrogens, typical changes in the vaginal smear and spontaneous temporary symptomatic relief (Papanicolaou and Shorr, 1936). Of even greater significance is the frequency with which symptoms may be alleviated or aggravated by changes in the extent of stress to which the patient is exposed (Shorr, 1940b).

Therapy with Androgens. The earliest reports of the effectiveness of androgens in relieving menopausal symptoms were those of Margiel and Zwilling (1936) and Mocquot and Moricard (1936a). The amount of androgens used by these investigators was much smaller than have subsequently been found necessary in later studies. Thus Mocquot and Moricard (1936a) observed complete relief in 4 of 8 patients receiving 5–10 mg. TAc at 1–2 week intervals. Their conclusions as to the influence of androgens on the menopausal syndrome were soon confirmed by Salmon (1937) who reported complete relief in one menopausal subject following the administration of 400 mg. TP in an average daily dose of 25 mg. and by Shorr, Papanicolaou, and Stimmel (1938) who observed the abolition of symptoms in 4 out of 5 menopausal women receiving 25 mg. TP daily for periods of approximately 21 days. Since then there have been numerous reports dealing with the use of androgens for this purpose (Birnberg, Kurzrok, and Livingston, 1938; Desmarest and Capitain, 1938; Laroche *et al.*, 1938; Kurzrok, Birnberg, and Livingston, 1939; Nathanson and Towne, 1939; Sharpey-Schafer, 1940; Silberman *et al.*, 1940; Berlind, 1941; Geist and Salmon, 1941; Salmon, 1941; Joël, 1942; Greenblatt, 1942c). They are characterized by the wide variety of dosage schedules employed and by the utilization of all the several clinically useful androgens and routes of administration. Dosages have ranged from as much as 100 mg. TP every other day for several weeks (Sharpey-Schafer, 1940) to as little as 20 mg. TAc or TP at monthly intervals (Desmarest and Capitain, 1938) with apparently equally good results. Greenblatt (1943a) has reported extremely satisfactory and prolonged relief from the implantation of pellets of TP totaling 50–400 mg. More recent reports have emphasized the convenience and effectiveness of MT in daily amounts of 10–20 mg. (Gusberg, 1945) or 30–50 mg. (Berlind, 1941).

Several paradoxical observations on the subjective effects of androgens warrant brief mention. Foss (1938) described the development of severe menopausal symptoms in the course of treatment of menorrhagia in a 40-year old woman, when amounts of 200 mg. TP were given weekly. A similar experience was reported by Sharpey-Schafer (1940) following the administration of 100 mg. TP every other day to two women with menorrhagia and to two normal men. In all four subjects flushes were subsequently abolished by means of estrogens alone or when given in conjunction with TP. These cases are to be distinguished from the several instances, reported by Foss (1938), Geist *et al.* (1940b) and Salmon (1941), in whom menopausal symptoms developed during the amenorrhea induced by TP after therapy had been discontinued.

In menopausal subjects treated with estrogens, whose hormonal requirements for symptomatic relief are such as to lead to uterine bleeding, androgens have been employed concurrently for the purpose of reducing or preventing this complication, presumably through a neutralizing effect on the endometrium. This desirable result was stated by Geist and Salmon (1941) to be achieved by a combination of estradiol benzoate and TP in a ratio of 1:10 by weight. Margolese (1944) obtained similar effects from the concomitant oral administration of 25 mg. MT and 1 mg. stilbestrol daily over periods of 4–6 months; no masculinizing effects were observed with these doses. Kurzrok and Rothbart (1942) found that the combination of 5–25 mg. MT and 2–3 mg. stilbestrol daily achieved excellent symptomatic results; although bleeding occasionally occurred, it was never excessive.

Discussion. The effectiveness of androgens in relieving menopausal symptoms in women is well established; the mechanisms are obscure. There is no correlation between symptomatic relief induced by androgens and alterations in urinary FSH excretion; indeed, relief may be experienced before any observable reduction occurs (Laroche *et al.*, 1938). The suggestion has also been made that androgens may act by exerting an "estrogenic" effect on the basis of several reports that their administration has led to typical estrogenic smears and increased urinary estrogen excretion. The weakness of this evidence has already been pointed out. As with estrogens, relief of the menopausal syndrome has been observed over a wide hormonal dosage range. These variations are, in part, a reflection of the difficulty of evaluating the therapy of a complex syndrome on a subjective basis; but to a larger degree they stem from the wide variation in the severity of the syndrome, individual differences in response to hormonal therapy and the influence of fluctuations in emotional stress.

The preferential use of androgens in a syndrome which may be of long duration must reside in whatever advantages androgens may possess over estrogens. In their favor is the fact that they cause no endometrial proliferation and, hence, no uterine bleeding. They may also have advantages as a temporary measure after operations for endometriosis, since they would insure the disappearance of residual endometrial implants. Their use would be preferable in young women with breast carcinoma with menopausal symptoms following castration. Their disadvantages arise from their induction of masculinizing phenomena when high doses are required for symptomatic relief. The continuous use of androgens in the menopausal syndrome would therefore be justified only when small, non-virilizing doses afford the desired relief.

The suggestion that the concomitant administration of androgens and estrogens will prevent bleeding and virilizing phenomenon, warrants more extensive study because of the potential value of this regime for patients who require such large amounts of estrogens for symptomatic relief that troublesome bleeding is inevitable. Except for the special situations discussed above, estrogens still appear to be the preferable hormone for the management of the menopausal syndrome and have the further advantage of being much less expensive.

6. *Libido*

The action of androgens in increasing sexual drive and responsiveness in women has led to their clinical use where such effects are therapeutically desirable.

Pathophysiology. The sexual drive and responsiveness in women is a complex reaction to which a variety of factors, psychological, emotional, physiological, anatomical, and hormonal, contribute. The concept that libido is solely dependent upon reproductive secretions is an untenable oversimplification of a complicated and subtle reaction. Indeed, there is an increasing recognition that most disturbances of libido arise from psychological and emotional maladjustments. Thus, during the reproductive phase, the quality of the ovarian function appears to have no relation to libido, as is evident from frigidity in women with normal ovulatory cycles and of normal sexual drive in anovulatory cycles and during periods of amenorrhea. Those variations in the quality and intensity of the sexual drive which may occur at different phases of the menstrual cycle and which have been attributed to specific hormonal alterations (Benedek and Rubenstein, 1939a,b) are more likely to be expressions of more general phenomena.

Changes in libido in the menopause are inconstant and, to a large extent, dependent on the previous sexual drive, the discomfort attendant on the menopausal syndrome, local involutionary changes of the external genitalia and vagina and the psychological reaction to the menopause as an episode in the aging process. When these result in a diminution in libido, it is a matter of general agreement that the most that can be expected from estrogenic therapy is a restoration to its premenopausal level. Those instances in which libido is heightened are attributable to psychological rather than hormonal factors.

Therapy with Androgens. Early in the clinical use of androgens with women the unexpected observation was made that they appreciably enhanced libido. In a group of menopausal women who first received estrous doses of estrogens and were then concomitantly given 25–50 mg. TP daily, libido and sexual response were significantly greater than that experienced with estrogens alone (Shorr, Papanicolaou and Stimmel, 1938). This was associated with marked vulval hyperemia and moderate enlargement of the clitoris. Since then the effect of androgens in increasing libido in women has been an almost universal observation (Loeser, 1940; Greenblatt, 1943a; Greenblatt and Wilcox, 1941; Greenblatt *et al.*, 1942; Salmon, 1941; Berlind, 1941; Salmon and Geist, 1943; Abel, 1945; Adair and Herrmann, 1946).

However, as might be anticipated from the variety of factors influencing libido, exceptions have also been noted (Silberman *et al.*, 1940; Greenblatt, 1943a). There are also several reports on the abolition of excessive libido by androgens. This was reported to have occurred in menstruating women receiving relatively small amounts of androgens premenstrually (Rubenstein *et al.*, 1940; Abarbanel, 1940b; Sturgis *et al.*, 1940).

Two extensive studies warrant detailed consideration. Greenblatt and Wilcox (1941), and Greenblatt *et al.* (1942), employed pellets of TP with a total weight of from 25 to 400 mg. in a group of 55 women with a variety of disorders, including functional bleeding and the menopausal syndrome, whose ages varied from 22 to 53. In 3 of 9 women with little or no previous libido, no change occurred; in the remainder, a moderate to marked increase. In 22 cases in which libido had been lost, it was uniformly restored. Of 10 with normal or excessive libido, 8 experienced no change, one an increase and one, previously nymphomaniacal, a decrease in sexual drive.

Salmon and Geist (1943) analyzed the effects of androgens on libido, classifying their subjects into 3 groups: (1) *primary frigidity*, which included those in whom libido had been previously absent; (2) *secondary* or "endocrine" loss of libido, secondary to the menopause or amenorrhea; and (3) subjects with *normal* libido. The dosage employed was 10–25 mg. TP 2 or 3 times weekly or 10–30 mg. MT daily, for periods of 6–8 weeks, or pellets of TP totaling 75–600 mg. In the first group of 29 subjects, there were 5 failures, 2 of whom had marital incompatibility, and 3, increased clitoral hypersensitivity without emotional response. Of the remainder, 20 developed normal or somewhat excessive libido and 4 were improved but failed to achieve orgasm. In those with excessive stimulation there was constant sensation of formication in the vulval area, most marked in the region of the clitoris. The clitoris and prepuce became hypersensitive and occasionally slightly hypertrophied. In the group secondary to the menopause or amenorrhea, all but 1 of 11 experienced increased libido and clitoral sensitivity, but no coital gratification. This latter circumstance was believed to be due to the dryness and tenderness of the vagina as a consequence of the involutionary changes induced by androgens; when the vaginal changes were relieved by sufficient estrogen to produce follicular smears, satisfactory intercourse followed. Twelve menopausal subjects with no improvement in libido following full estrous doses of estrogens were then concurrently given 25 mg. TP three times weekly. Within three weeks, 4 had developed normal libido, 2 excessive, 1 a slight increase, while 1 remained unchanged. Thus, a combination of estrogen and androgen exerted their optimal effects on libido and sexual response when the smear was at full estrus. In the group of women with normal libido, 30–50 mg. TP weekly, 20–50 mg. MT daily or 300–600 mg. TP in pellets, led to a progressive increase in libido in all but 1 of 10; in 4 subjects receiving TP, libido became excessive. Of 15 patients receiving pellets, 3 were unchanged, 5 had moderate, and 7 excessive stimulation. The authors attributed most of the failures to the presence of psychic factors in the genesis of the frigidity; however, they did not state how they were able to rule out such factors in cases of so-called "primary" frigidity.

Finally, mention should be made of the experience of Groome (1939) with the local application of a TP ointment to the vulva, which was limited to a single case. A total of 7.6 mg. TP was applied locally over a period of 22 days. There was an enlargement of the clitoris, but no other changes in the genitalia. Increased libido and successful orgasm were achieved.

Discussion. A number of explanations have been offered for the effect of androgens in increasing libido in women. The complexity of the factors contributing to the sexual drive suggests that multiple influences are involved. The most obvious are those which might result from the changes induced by androgens in the external genitalia, which range from increased sensitivity to engorgement and hypertrophy of the clitoris and extreme vulval hyperemia. These local changes are accompanied by an awareness of sensations which are usually secondary to sexual stimulation and which may consequently initiate the sequence of events leading to increased libido and sexual responsiveness. However, the local vulval changes may not provide the full explanation for the enhancement of libido in all cases and other factors may be contributory, such as the increased well-being induced by androgens in the menopausal syndrome, functional bleeding, endometriosis and carcinoma.

Those occasional instances of excessive libido associated with premenstrual tension, which were reduced by androgens, may owe this effect to the amelioration of the premenstrual tension as well as to the psychotherapeutic benefits of medical care.

The actions of androgens on sexual behavior are of considerable therapeutic significance for patients in whom frigidity is at the center of a serious marital or psychological maladjustment. The ideal use of androgens for this condition would be afforded by a regime whose action was confined to this one effect; for this reason the effectiveness of the local application of TP ointments warrants further exploration.

It should be borne in mind, however, that the effect on libido may not be desirable in all instances, since increased libido without normal channels for satisfaction may become a serious individual problem and lead to psychic trauma.

7. *Functional and Pathological Disorders of the Breast*

a. Breast Pain and Benign Lesions. The dependence of breast growth and development on hormonal factors has led to the inference that a variety of signs and symptoms referable to the breast may result from hormonal disturbances and be susceptible of management by hormonal agents. For these reasons a variety of hormones, including estrogens, progesterone and prolactin as well as androgens, have been given a therapeutic trial. The appropriateness of the use of these hormonal agents should be projected against what is known of the normal and pathological physiology of the breast (Nelson, 1936; Turner, 1939).

Pathophysiology. Although there are species differences, normal spontaneous breast development is dependent upon normal ovarian activity and the elaboration of estrogen and progesterone. The former hormone exerts its effects predominantly on the growth of the ducts and acts synergistically with progesterone to lead to lobule-alveolar development. Analysis of the factors participating in breast development by means of the administration of hormones to ovariectomized and hypophysectomized animals have shown that, although the major contributing factors are estrogens and progesterone, other hormonal influences may also be involved. The synergizing effects of prolactin in estrogen-induced breast growth have been demonstrated (White, 1943) and there is evidence of a less specific character, that lipid-soluble and as yet inadequately characterized pituitary factors, termed mammogens, may also be involved (Turner, 1939; White, 1943). The potential participation of adrenal cortical factors, specifically, desoxycorticosterone, has also become apparent from animal experimentation (Speert, in press).

Of particular relevance to the present discussion are those studies relating to the action of androgens on the mammary gland. The mammotropic action of androgens has been demonstrated in a number of species, the studies on the monkey being of special interest (Folley *et al.*, 1939; Van Wagenen and Folley, 1939a,b; Speert, in press). Although some authors would limit the effects of androgens to dilation of the ducts and to acinar formation without increase in the lobule-alveolar mass, Speert (in press) consistently observed distinct enlargement of the lobules, dilation of the acini, and stromal changes similar to the edema of the interlobular connective tissue induced by progesterone. Priming of the castrate monkey with estrogen prior to the administration of TP potentiated the effect of the androgen. One additional action of androgens was observed by Folley *et al.* (1939) and Van Wagenen and Folley (1939b) and Speert (in press), namely, an irregular heaping up of the epithelium resembling intraductal papilloma. In the human, gynecomastia has been observed in a number of males under prolonged therapy with MT (McCullagh and Rossmiller, 1941).

The spontaneous development of the breast in all species including the primates is dependent on the initiation of ovarian secretory activity. Once breast development is established in monkey and man it is not a static phenomenon, but exhibits cyclic alterations in relation to the menstrual cycle. In the rhesus monkey Speert (1941) has described the regular occurrence of periodic alterations during ovulatory cycles. These consist of a premenstrual increase in size and vascularity of the lobules and a dilation of the acini during the premenstrual and menstrual phases; thereafter, regression takes place and persists until the next premenstruum. These changes were absent in anovulatory cycles.

Although there is less uniformity in the description of the periodic changes in the human breast, due in large measure to the difficulties in obtaining material under ideal conditions for study, the weight of evidence favors the existence of similar rhythmic changes in relation to the menstrual cycle (Rosenburg, 1922; Pollano, 1924; Lewis and Geschickter, 1934; Foote and Stewart, 1945,a,b). During the luteal phase there have been described not only marked growth in the lobule-alveolar system and dilation of the acini, but increases in vascularity and significant stromal edema; and following menstruation, a similar regression to that observed in the monkey.

The benign clinical disorders of the breast may be classified into two groups, those associated with periodic functional and reversible changes and those with varying degrees of pathological alterations of a noncancerous nature. The first includes the painful swelling and tenderness of the breast which occurs so commonly during the premenstrual phase and disappears with, or shortly after, the onset of menstruation (Taylor and Waltman, 1940; Taylor, 1942). Such histological studies as are available of this uncomplicated syndrome reveal the absence of pathological changes except for an increase of the interstitial fluid of the periacinar spaces and loosening of the connective tissue. It may be inferred from the studies cited above, that these stromal changes are also accompanied by the growth of the lobule-alveolar system and increased vascularity characteristic of the premenstrual breast during ovulatory cycles.

The differentiation of breast pain arising from purely physiological and reversible premenstrual phenomena from that in which pathological processes are also involved, is admittedly difficult. Indeed, some authors suggest that the former syndrome may actually represent an early stage of adenofibrosis (Taylor, 1942). The latter condition usually has as its earliest manifestation an area of induration limited to the upper outer quadrant. In its advanced stage, the fibrous change may involve the entire breast and assume a discoid shape with a well-defined edge. The histology is characterized by extensive connective tissue proliferation resulting in a distortion of the glandular fields and, not infrequently, by areas of acinar proliferation. Adenofibrosis is usually accompanied by premenstrual exacerbations of the breast pain and tenderness, attributed to the increased intramammary tension associated with increased vascular engorgement, interstitial edema and glandular development characteristic of this phase of the menstrual cycle.

A second type of abnormality manifests itself by intermittent or continuous discharge of a serous or milky secretion from the nipple, and, not infrequently, by cyclical or acyclical pain or swelling. The underlying histological picture is usually that of dilation of the ducts by stagnant secretion, periductal edema, lymphocytic infiltration and, in some instances, glandular epithelial hyperplasia. The dilated ducts may be felt as cord-like structures radiating from the nipple. To this condition the name non-puerperal mammary secretion has been given (Taylor, 1942; Nathanson, 1946a,b). Combinations of adenofibrosis and non-puerperal mammary secretion are not uncommon.

A third category of nodular diseases of the breast, frequently unassociated with pain or tenderness, include the benign single or multiple cysts with or without epithelial hyperplasia (Reclus' disease); and multiple cysts and epithelial hyperplasia accompanied by papillomatous changes (Schimmelbusch's disease) usually regarded to be of precancerous nature.

For a comprehensive review of this subject the reader is referred to papers by Taylor (1942), Nathanson (1944, 1946a,b) and Foote and Stewart (1945a,b).

The use of androgens in these disorders is based on the dependence of the breast for growth and development on the ovarian hormones, the influence of androgens on ovarian secretory activity and on the frequency with which these breast disorders are associated with derangements of menstrual function and pelvic disease. Thus, there is a high incidence of infertility and oligomenorrhea in adenofibrosis in spite of the presence of normal endometrial patterns. Adenofibrosis is also commonly associated with a type of pelvic disease usually termed chronic pelvic congestion or chronic parametritis (Taylor, 1942). Non-puerperal mammary secretion with its highest incidence in previously fertile women is nevertheless accompanied, in many cases, by a prolongation of the intermenstrual interval and atypical endometrial patterns. Direct evidence of the influence of hormonal factors on both disorders is their tendency to subside after the menopause. On the other hand, cystic disease of the breast is much more common after the age of reproductive activity.

Therapy with Androgens. The evaluation of the therapeutic effectiveness of androgens for this group of breast disorders is complicated by the frequency with which spontaneous improvement occurs and the usual absence of diagnostic criteria other than palpation. Furthermore, even the information provided by biopsies may be unreliable since such specimens are rarely representative of the state prevailing in the whole breast.

The first report on the therapeutic effectiveness of androgens in this group of breast disorders was that of Desmarest and Capitain (1937a,b). They reported beneficial results in 16 of 17 cases with a variety of palpable breast changes associated with chronic or premenstrual pain. In the following year they (Desmarest and Capitain, 1938; Desmarest, 1938) reported similar favorable results in an additional group of cases receiving 5–10 mg. TAc daily from the tenth day of the cycle to 1 or 2 days before the next flow. These reports were soon confirmed by Turpault (1937) who used 10–30 mg. TP 3 days before menstruation and by Bender (1937) who employed a total dosage of 15–80 mg. TP during the second half of the cycle. Loeser (1938a) employed larger doses, totaling 600 mg. TP given over a period of 3–4 weeks, with symptomatic relief and, in one case, the disappearance of the masses. Other studies describing the relief of breast pain following the premenstrual administration of 15–40 mg. TP or the midmenstrual administration of 40–50 mg. TP are those of Tailhefer (1938), Douay (1938), Audit (1938), Evelbauer (1939) and Geist (1941). Foss (1938) described the successful treatment of nonpuerperal mammary secretion associated with painful breast nodules. In all these studies, except that of Loeser (1938a), the amounts of androgens employed were too small to have exerted an appreciable effect on ovarian function.

The relation of dosage to therapeutic effects was first recognized by Atkins (1940) who reported relief in only 50% of patients with painful breasts who received less than 400 mg. TP over 4 weeks, whereas 86% receiving 400 mg. TP or more for the same period were benefited. There was, however, a high incidence of virilizing effects in the latter group. This study is unique in its utilization of biopsies before and after treatment, none of which, regardless of dosage, showed significant changes following androgenic therapy. The temporary nature of the benefit was evident from the frequency of recurrences in a number of the patients whose progress could be followed.

The evaluation of the relief of breast pain by androgens is further complicated by the participation of psychological factors (Spence, 1939). Of 24 patients, 5 of whom had pain in association with nodular breasts, 13 experienced relief following the injection of sterile olive oil. In the group refractory to placebos, which comprised a large number with nodular breasts, nearly all were relieved by the biweekly administration of 25–100 mg. TP for several months. In 12 subjects with nodular breasts, the nodules disappeared in 3 and decreased slightly in 5; in 2, new nodules appeared. Spence (1940) subsequently reported that the daily inunction of the breasts with an ointment containing 3–10 mg. TP per application, provided relief in 8 patients, only 2 of whom had responded to a non-medicated ointment.

There remain for consideration the more detailed studies of Nathanson *et al.* (1942) and Taylor (1942). The series studied by Nathanson and his associates comprised 9 patients with adenofibrosis and 21 with non-puerperal mammary secretion. The dosage regimes varied; in most cases 10 mg. TP were given every other day for the 2 weeks prior to menstruation; in a few cases therapy was continued throughout the cycle. In an occasional case the dosage unit was 25 mg. TP. The average monthly dosage ranged from 75–150 mg. TP per month, with a few cases receiving 250 mg. per month. In some cases inunctions of 2–4 mg. TP were applied to each breast before retiring. Of the 9 patients with adenofibrosis, 6 experienced complete and 3 partial relief of pain; in 7 there was also some improvement in physical signs. Of 21 patients with non-puerperal mammary secretion, 12 obtained complete and 6 partial symptomatic relief; secretion was abolished in 13 and lessened in 5. All physical signs disappeared in 3 and diminished in 15. However, the temporary character of these effects was evident from the usual recurrence of signs and symptoms within 6 months after therapy was discontinued. Taylor's (1942) series of 11 patients included 10 with adenofibrosis, in 2 of whom there was an associated mammary secretion; the remaining case had non-puerperal mammary secretion. They were treated for 1–4 months with an average monthly dose of 175 mg. TP, and a total average dose of 350 mg. During the period of treatment, 8 experienced partial or complete relief of pain, 3 a reduction or disappearance of the nodularity and in one the mammary secretion was abolished. However, the late effects, *i.e.*, 2 years after hormonal treatment, were no better than the spontaneous improvement observed in a control series.

Discussion. Despite the difficulties introduced in the evaluation of any therapeutic regime by psychological factors, spontaneous remissions and diagnostic uncertainties, it may be concluded that androgens achieve considerable subjective relief and, less regularly, objective changes in the syndrome of breast pain, with or without associated benign pathological changes. The favorable results appear to be of a temporary character; following discontinuation of therapy, the recurrence rate is high and the end results not superior to those that occur spontaneously. In most studies, the amounts of hormone found adequate to relieve pain were too small to produce any detectable changes in ovarian function. The mechanism by which these effects were achieved still awaits clarification. Indeed, relief of breast engorgement from the use of androgens would not have been anticipated from studies in monkeys which have shown that androgens, particularly in the presence of estrogens, produce lobule-alveolar growth and interstitial edema comparable to that induced by progesterone. Furthermore, androgens have produced gynecomastia in males. Whether the temporary palliation of these breast disorders will continue to justify their treatment by androgens will depend on the extent to which the symptoms are disabling and the availability of more effective measures.

b. The Painful Postpartum Breast and Lactation. The suppressive effects of androgens on lactation in rodents have led to their clinical use for this purpose as well as for the relief of the painful postpartum breast.

Pathophysiology. The course of pregnancy is associated with a progressive enlargement of the breast to which all the mammary components contribute. There is marked hyperplasia of the duct and lobule-alveolar systems, an increase in the vascular and lymphatic supply, and in the secretory activity (Turner, 1939; Geschickter, 1943). Lactation is deferred until the third to fifth day postpartum and is believed to result from the stimulation of the breast by prolactin, whose secretion is suppressed by the high levels of estrogen which prevail during pregnancy and is then released by the precipitous drop in estrogen titer following delivery. The suckling reflex is a major factor in the initiation as well as in the maintenance of lactation. The vascular and lymphatic stasis in the breast on the third or fourth day postpartum is considered responsible for the breast pain of this period, which subsides with, or shortly after, the onset of lactation in nursing mothers, to recur should nursing be interrupted. In non-nursing mothers, painful engorgement persists for variable lengths of time beyond the onset of lactation. The use of androgens for the purpose of influencing both painful engorgement and lactation stems from the demonstration by Robson (1937c) in mice, and Folley and Kon (1938) in rats, that androgens inhibited lactation in both species.

Therapy with Androgens. The first report of the clinical use of androgens for the purpose of inhibiting lactation was that of Kurzrok and O'Connell (1938). Patients were given 10–25 mg. TP twice daily for 1–5 days, treatment being initiated on the first to the eleventh day postpartum, usually on day 3. This regime relieved all symptoms of breast engorgement usually associated with the interruption of nursing and led to the inhibition of lactation, without further discomfort, in 19 of 21 patients. Complete relief usually was achieved within 48 hours and optimal results were obtained with doses of 25 mg. twice daily. This experience has been duplicated by numerous other investigators using a variety of dosage regimes as well as times for the initiation of treatment. The majority have employed a total dosage of 50–125 mg. TP or its equivalent of MT, starting medication 36–72 hours postpartum (Peralta Ramos and Colombo, 1938; Portes *et al.*, 1939; von Konrád, 1939; Birnberg *et al.*, 1940; Beilly and Solomon, 1940; Siegler and Silverstein, 1940; Hellman and Ciner, 1941; Berlind, 1941; Kuschner, 1942; Lass, 1942; Duckman and Turino, 1942; Jeppson *et al.*, 1942). However, equally effective results were reported with as little as 10 mg. TP (Abarbanel, 1939), or 8 mg. TP by daily inunction (Fleischer and Kuschner, 1941).

At least one investigator reported that the optimal time for the initiation of androgenic therapy was on the third to fourth day postpartum, the usual time of onset of the painful engorgement, and that it was impossible to prevent its appearance by instituting therapy immediately after delivery (Duffy and Corsaro, 1941).

Although Kurzrok and O'Connell (1938) and most other investigators interpreted the effect of androgens on the postpartum breast as an inhibition of lactation, Abarbanel (1941) showed this interpretation to be incorrect. No inhibition of lactation occurred in those mothers who continued to nurse. This observation on the failure of androgens to inhibit lactation was amplified by Preissecker (1940) who administered 10–25 mg. TP daily, for a total dose of 35–105 mg., beginning on the tenth day postpartum, to 11 nursing women with well-established lactation. In 5, an increase in milk secretion was observed during therapy; in the remainder the amount of secretion was unaltered. Much larger amounts of TP, totaling 250–500 mg. and given over a period of 24–72 hours after lactation had been established, were also without effect on the milk secretion of nursing mothers (Abarbanel, 1941). Similar results were obtained with the administration of 200–500 mg. MT over the same period of time. This author was unable to inhibit the onset of lactation in nursing mothers by the administration of 200–300 mg. TP for 2–5 days, the hormonal treatment starting shortly after parturition. These results have also been confirmed by Stewart and Pratt (1941) and Rutherford (1942).

Discussion. There is general agreement as to the effectiveness of androgens in relieving painful breast engorgement of the puerperium and in non-nursing mothers, thereby permitting a comfortable spontaneous regression to the non-secretory state. Androgens exert their effect by relieving the painful breast engorgement and are without direct influence on lactation, which may be initiated by suckling despite the continued administration of large amounts of androgens and, once established, is unaffected as long as nursing is continued. The relief is believed to result from a reduction in vascular stasis and interstitial fluid though no direct evidence for this inference has yet been provided.

This method of therapy is regarded by many workers as more effective than the usual conservative measures employed. It is also considered by many to be preferable to the use of estrogens which, although equally effective in relieving pain, have certain disadvantages, such as the occasional induction of withdrawal bleeding (Clahr, 1941) and the frequency with which congestion and lactation reappear within a few days after therapy is discontinued (Walsh and Stromme, 1944).

c. Breast Carcinoma: Primary and Metastatic. The growing appreciation in recent years of the relation of hormonal factors, particularly estrogens, to carcinoma of the breast has drawn attention to the possible usefulness of androgens for this condition.

Pathophysiology. A discussion of the possible role of hormones in the genesis of breast carcinoma is beyond the scope of this review. Certain observations, however, may be considered which are relevant to the use of androgens in its management. In 1896 (a,b), Beatson called attention to the amelioration of signs and symptoms of carcinoma of the breast following removal of the ovaries, in 2 young women with advanced carcinoma of the breast; and subsequent experience has confirmed his findings not only with respect to the primary lesion but also to the bony metastases. Direct evidence of the influence of estrogens on neocarcinogenesis was provided by the experiments of Lacassagne (1932) which showed a striking enhancement of the incidence of mammary cancer in male mice of strains in which mammary cancer was almost regularly present in the female. The carcinogenic effects of estrogens on the mammary gland have since been established for a number of species of experimental animals, of which the monkey remains an exception. In man, the consensus is that mammary cancer does not result from the administration of estrogens; indeed the studies of Foote and Stewart (1945a,b) have shown that the glandular changes induced by estrogenic stimulation of the breast differ fundamentally from those observed in neoplasia. Nevertheless, this does not exclude endogenous alterations of hormonal metabolism involving estrogens and other steroidal hormones as contributing factors. The use of androgens in carcinoma derives support from the rarity of breast carcinoma in the male and the demonstration by Nathanson and Andervont (1939) and Lacassagne and Raynaud (1939) that the administration of androgens to strains of mice with a high incidence of mammary carcinoma in the female appreciably reduced the incidence.

Therapy with Androgens. Only a few reports have appeared relating to the clinical use of androgens in the treatment of carcinoma of the breast and their metastases in women; the number of patients and the period of study have been too limited to permit any conclusions as to the contribution which androgens may make. The first reports were those of Ulrich (1938, 1939,a,b,c) who used small doses for short periods of time with "favorable results" in two cases, and a somewhat larger series of Loeser (1939a,b; 1940; 1941) who employed amounts of TP, 700 mg. over 2 months, sufficient to suppress the menstrual cycle. In 3 of 5 cases treated with several such courses, or by large amounts of TP in pellets, regression of skin and axillary recurrences was described in 4 of 5 patients; their subsequent history was never reported. Both investigators were struck with the relief of pain experienced. Farrow and Woodward (1942) and Farrow (1944) treated 33 cases of breast carcinoma with skeletal metastases with 5–25 mg. TP 1–3 times weekly for a total of 10–12 doses. Relief of pain was experienced by half of the patients, but there was no associated clinical or roentgenographical evidence of control of the disease. Three patients received larger doses of TP (470 mg. in 7 days, 400 mg. in 8 days, 500 mg. in 10 days); all developed hypercalcemia and hypercalcinuria which were attributed to increased osteolytic activity of the bony metastases. Fels (1944) treated 3 cases with suppressive doses of androgens and observed an improvement in general well-being and in the calcification of the skeleton, as well as some local tissue repair. Prudente (1945) compared the survival and recurrences in a group of 63 women receiving no TP after mastectomy with an equal number given TP continuously postoperatively. The dosage varied directly with the degree of malignancy as judged histologically, and ranged from 25 mg. TP weekly in low grade malignancy to 75–100 mg. weekly in the most malignant group. He reports a somewhat higher survival rate and a lower incidence of recurrence in the group treated with androgens.

The most recent reports are those of Adair and Herrmann (1946) and Herrmann, Adair and Woodward (1947). The first included 9 cases with soft part disease and 3 with osseous metastases; the second added 11 additional cases with osseous metastases. In both studies the dosage regime and the duration of therapy varied from 200 mg. TP daily or twice weekly to 25 mg. TP thrice weekly. Total dosage varied from 1100 mg. to 9350 mg. in 7–35 weeks, depending upon the duration of therapy, most cases receiving from 3000–5000 mg. in all. The therapeutic results of the two papers may be summarized as follows. In 7 of the 9 patients with soft tissue disease and 3 of the 14 with osseous metastases the progress of the disease was uninfluenced. Of the 14 patients with osseous metastases, 2 showed significant temporary improvement but subsequently deteriorated and 3 were not benefited. The remaining 9 were maintaining their symptomatic improvement at the time of publication. Their course was marked by relief of bone pain, which was accompanied in the majority of cases by X-ray evidence of recalcification and by an elevation in alkaline phosphatase and a fall in serum calcium. In several cases pain recurred on discontinuation and disappeared on reinstitution of therapy. The most rapid relief of pain was achieved with dosages of 100–200 mg. 2–3 times weekly. In all, masculinizing phenomena were prominent and in all, weight gain and improvement in well-being were striking, although limited to the period of therapy.

A single case of Bolger (1946) which was surgically castrated and treated with small amounts of MT is impossible to evaluate.

Discussion. Temporary benefit may result from the use of androgens in women with carcinoma of the breast with or without metastases. These effects are more pronounced in those with osseous metastases and take the form of relief from bone pain and an increase in weight and well-being. The relief of pain is often accompanied by evidence of bone repair. The increased well-being and weight gain are ascribable to the metabolic effects of androgens on nitrogen metabolism. Improvement is more likely to occur with the use of large doses of androgens whose virilizing effects are of minor importance under these circumstances.

The evidence to date does not permit any conclusions as to the role which androgens may play in the management of breast carcinoma and their metastatic lesions, but there is no doubt as to their temporary palliative effects.

8. Disorders Related to Pregnancy

a. Nausea and Vomiting. A single report describes the use of androgens in the management of nausea and vomiting occurring in early pregnancy (Shute, 1941). Thirty-five women, in whom these symptoms of unspecified intensity were associated with estrogen titers considered to be abnormally high by the author's standards, were given 10 mg. TP at 3-day intervals with complete relief of symptoms in 80%. A group of

15 patients with similar symptoms, but with low estrogen titers, were treated with estrogens with equally good results. The absence of well-characterized hormonal changes in association with the nausea and vomiting of early pregnancy, the large psychological and emotional element involved, and the reported effectiveness of a wide variety of therapeutic measures, make it difficult to evaluate the specificity of these therapeutic effects.

b. After Pains. It is generally held that after pains arise from firm and sustained uterine contractions which occur postpartum in response to the stimulus of the intrauterine contents and are comparable to the cramp-like pains of dysmenorrhea. They are usually adequately managed by analgesics. However, the benefit observed with androgens in dysmenorrhea suggested their use for after pains as well. Prompt and sustained relief has been reported to follow the parenteral administration of 10 mg. TP at or near the time of delivery (Abarbanel, 1939) or 12–24 hours postpartum (Mandy and Mandy, 1940). In 2 series of cases in which TP was used to suppress lactation the patients were also free of after pains (Kurzrok and O'Connell, 1938; Fleischer and Kuschner, 1941). Whether the use of androgens for this condition has advantages over its customary management by analgesics will be determined by further experience.

9. Metabolic and Endocrine Disorders

a. Cushing's Syndrome. The use of androgens in the management of Cushing's syndrome derives from the evidence of widespread protein depletion in various tissues of the body and the well-established property of androgens to cause the storage of proteins in animals and man. The consequences of this protein depletion in Cushing's syndrome are apparent in the widespread osteoporosis, which is believed to result from a reduction of the organic matrix of bone on which deposition of calcium depends, in the atrophic changes in the skin and the reduction of muscle mass and strength with the associated creatinuria. These alterations in protein metabolism are believed to result from a preponderance in the organism of adrenocortical hormones which promote gluconeogenesis from protein over those which make for nitrogen storage. These inferences derive from the demonstration of the nitrogen storing properties of androgens in animals (Kochakian and Murlin, 1935) and in man (Kenyon *et al.*, 1938); and from the animal experiments of Long *et al.* (1940) which have revealed the enhancement of gluconeogenesis from protein under the influence of corticosterone. Urinary hormonal assays (Anderson *et al.*, 1938; Weil and Browne, 1940) have shown the regular presence of excessive amounts of corticosteroids of the type which promote gluconeogenesis. Urinary 17-ketosteroids are usually slightly and sometimes significantly elevated, although it still remains uncertain just what is the exact hormonal composition of this mixture of steroidal hormones which develop a color with the Zimmerman reaction (Dobriner, personal communication).

This hormonal imbalance would appear to be susceptible of correction by the administration of amounts of androgens sufficient to overcome the preponderance of adrenocortical hormones favoring gluconeogenesis, and a number of reports have described in more or less detail the metabolic and symptomatic effects which follow the use of androgens in Cushing's syndrome. The comprehensive metabolic studies of Albright, Parsons *et al.* (1941) and Albright (1942–1943) may be summarized as follows. Administration of 25–50 mg. TP a day resulted in a significant retention of nitrogen and phosphorus which was manifested early in the course of treatment, and a subsequent progressive increase in calcium storage accompanied by a rise in serum alkaline phosphatase. These changes were associated with an increase in muscle mass and disappearance of creatinuria, as well as an improvement in the texture of the skin. The bone changes were usually not discernible by X-ray but were manifested symptomatically by freedom from pain in the face of increased activity. All metabolic changes were reversed on discontinuation of therapy. Methyl testosterone produced similar changes except for its usual enhancement of creatine excretion, but appeared to be somewhat less effective than TP in the doses used. The delayed sugar tolerance curve and insulin resistance commonly seen in this disease were often corrected. Estradiol benzoate, administered to 2 patients with Cushing's syndrome, did not significantly alter the nitrogen, phosphorus or calcium balances, nor did it, in conjunction with TP, increase the beneficial effects achieved with that agent alone. This was contrary to the findings of Shorr (1942) of an augmentation of nitrogen retention in a case of Cushing's syndrome resulting from the combined treatment with both hormones.

Similar changes in nitrogen and creatine metabolism were observed by Deakins *et al.* (1944) to follow the administration of 25 mg. TP for 17 days and 40 mg. MT for 16 days. No clinical improvement accompanied these brief courses of therapy. A similar retention of nitrogen, improvement in glucose tolerance curves and some recalcification of the spine followed the daily administration of 25 mg. TP for 123 days to a 30-year old woman (Perloff *et al.*, 1943). Virilizing effects, which were usually present and often extreme, present an undesirable complication, particularly since prolonged therapy may be required. It seems likely that this complication can be avoided by combined therapy with androgens and estrogens, as suggested by Shorr (1942). Increasing experience with such combined therapy in Cushing's syndrome and postmenopausal osteoporosis suggests that this regime may not only be additive with respect to nitrogen, phosphorus and calcium storage but significantly reduces the virilizing effects (Carter and Shorr, unpublished data). We may conclude that TP alone, or in combination with estrogens, corrects many of the metabolic derangements characteristic of Cushing's syndrome and induces increased well-being during the period of therapy. There is as yet insufficient clinical experience to permit conclusions as to the effects of these agents on the ultimate outcome of this disease.

b. Postmenopausal Osteoporosis. The defect in organic bony matrix formation in Cushing's syndrome is also considered to be operative in the genesis of postmenopausal osteoporosis. Albright *et al.* (1940, 1941) have described the beneficial effects of estrogens on the storage of calcium and phosphorus in this latter condition. The possible therapeutic effectiveness of androgens as well, is suggested by their superiority over estrogens in effecting protein storage as well as by the retention of calcium which results from their administration to patients with Cushing's syndrome. The advantages of the combined use of androgens and estrogens in the management of the osteoporosis of Cushing's syndrome has just been pointed out. In postmenopausal osteoporosis, this regime has the added advantage of reducing or eliminating the undesirable bleeding which may be expected to follow either prolonged administration of estrogens or their discontinuation in women with intact uteri (Carter and Shorr, unpublished data), particularly since the large amounts of estrogens required for nitrogen and calcium storage can be expected to lead to endometrial hyperplasia.

c. Simmonds' Disease. MT was used by Werner and West (1943) in 2 cases of Simmonds' disease, with cachexia and definite pituitary lesions, in doses of 100 mg. daily. This therapy achieved marked nitrogen retention associated with a gain of weight and a temporary creatinuria, which subsided on discontinuation of therapy. Both patients experienced marked subjective improvement, renewed vigor, increased strength and libido and redevelopment of secondary sexual characteristics. Williams and Whittenberger (1942) and Williams *et al.* (1945) treated 6 patients, including 4 women, with Simmonds' disease and proved pituitary lesions with TP, MT or T pellets, with similar results as regards increased strength, energy and well-being. Metabolic studies in 2 cases showed storage of nitrogen, potassium and sodium during the daily administration of 25 mg. TP or 60 mg. MT. There was some reduction in creatine-creatinine excretion with the former compound and inexplicable enhancement of creatinine excretion with the latter.

d. Acromegaly. Schrire and Sharpey-Schafer (1938) studied the effects of TP as well as estrogens on the urinary excretion of creatine and creatinine in 2 females and 2 males with acromegaly. Both hormones in large doses (10 mg. estradiol benzoate or 100 mg. TP daily) led to a rapid and striking reduction in the urinary excretion of both creatine and creatinine to levels characteristic of the normal individual. In one instance there was an exacerbation of headache during the administration of both hormones, which was attributed to a temporary increase in the size of the pituitary gland.

The exact mode by which this interesting effect was achieved requires elucidation, but it would appear that the effects were not due to a direct action of the hormones on the creatine-creatinine metabolism of muscle but rather mediated in some manner through the pituitary. That this effect may not be regularly induced is evident from the failure of as much as 10 mg. estradiol dipropionate daily to influence creatine-creatinine metabolism in an acromegalic male (Albright, Reifenstein, and Forbes, 1946).

e. Pituitary Dwarfism. Wilkins, Fleischmann, and Howard (1941) and Wilkins and Fleischmann (1944) report far greater effects on growth and the development of the secondary sexual characteristics in certain cases of pituitary dwarfism in young girls, when MT in daily doses of 10–25 mg. were given in conjunction with 1 mg. stilbestrol, than from the use of estrogens alone. No significant virilizing effects were observed with this regime. This interpretation, however, is predicated on the unsupported assumption that the dose of stilbestrol employed was sufficient to produce the full somatic developmental effects inherent in complete estrogenic replacement therapy.

f. Addison's Disease. The uniformity with which Addison's disease in both sexes is accompanied by a profound reduction in urinary 17-ketosteroids, as well as in the corticosteroids influencing carbohydrate and electrolyte metabolism, would suggest that more adequate replacement therapy might be achieved in this disease by the use of androgens in conjunction with desoxycorticosterone acetate or adrenal cortical extracts. That androgens are as capable of inducing gain of weight and the storage of nitrogen, sodium, potassium, phosphorus and chlorides in Addison's disease as in normal subjects has been established by Kenyon *et al.* (1938) and Thorn and Engel (1938). The earliest report of the effects of TP in Addison's disease is that of Marañón (1938). TP was given to two men and one woman for periods of five weeks; all experienced significant subjective improvement and a gain in weight, without an alteration in blood pressure.

The careful study of Talbot, Butler, and MacLachlan (1943) of the metabolic and subjective effects of androgens in an 8 year old girl with

Addison's disease is of particular interest. Except for two short periods, she was maintained on desoxycorticosterone acetate and was given extra salt throughout. Metabolic studies during the daily administration of 90 mg. MT or 25–50 mg. TP resulted in a gain of body weight, a diminution in the urinary excretion of nitrogen, potassium and sodium and a marked fall in serum potassium concentrations. This last observation is confirmatory of previous studies in normal subjects; and it is of interest that a reduction of serum potassium to the very low level of 0.7 m. eq./l. was unaccompanied by untoward symptomatic or electrocardiographic changes. When desoxycorticosterone acetate was omitted, MT induced nitrogen storage but failed to prevent loss of weight and the recurrence of symptoms suggestive of acute adrenal insufficiency. On the other hand, 50 mg. TP daily during desoxycorticosterone withdrawal served not only to enhance nitrogen storage, but also to maintain weight and well-being. Methyl androstenediol in doses of 50 mg. a day orally achieved much less nitrogen retention and had the disadvantage of irritating the gastrointestinal tract. During the 10 months study period in which androgens were employed, there was a 3 year advance in skeletal development, a definite increase in musculature, slight lowering of the voice, some growth of pubic hair and slight hypertrophy of the clitoris.

Somewhat similar results, as regards subjective improvement, weight gain and retention of nitrogen, potassium and sodium were observed by Williams *et al.* (1945) in 5 patients, 2 of whom were women. A variety of dosage levels of MT and TP as well as T pellets were employed. The daily administration of 40 mg. androstandiol intramuscularly and of 40–80 mg. methyl androstandiol orally were also generally associated with increased strength and well-being; however, these symptomatic changes were somewhat less regular and usually unassociated with significant changes in nitrogen or electrolyte metabolism. Our own experience with the supplemental use of androgens for Addison's disease in both sexes, would suggest that it is particularly applicable to those patients whose response to desoxycorticosterone acetate has not been entirely satisfactory. The adjuvant use of androgens in Addison's disease would appear to warrant more extensive clinical trial on theoretical grounds and from its direct effect on nitrogen and electrolyte metabolism.

g. Graves' Disease. The first report of the possible usefulness of androgens in the treatment of Graves' disease was that of Loeser (1938b); 850 mg. TP given over 2 months was stated to diminish the thyrotoxic symptoms in a 14 year old girl with Graves' disease. A more detailed study of Kinsell, Hertz and Reifenstein (1944) relates particularly to the influence of TP and MT on nitrogen balance, creatine excretion and other metabolic factors in 3 subjects with Graves' disease, 2 of them women, who received 12.5–100 mg. TP or 50–100 mg. MT daily. TP induced a markedly positive nitrogen balance and weight gain comparable to that in normal subjects, and MT the same changes but to a lesser degree. In one case, there occurred a reduction in the urinary calcium excretion; and in another, serum potassium levels were lowered to the same extent as in normal subjects. The effects of both androgens were studied on urinary creatine; TP abolished, and MT augmented, the creatine excretion which is a regular accompaniment of Graves' disease, and which is generally regarded as directly related to the muscle weakness and myopathies of this disease. None of the subjects were on creatine-creatinine-free diets and one received potassium iodide which usually eliminates creatinuria in Graves' disease, circumstances which complicate the evaluation of the creatine effects observed. There is, nevertheless, no doubt of the validity of their observations that TP abolishes the creatinuria of this state. The clinical course appeared to be somewhat ameliorated by TP and worsened by MT. These changes were not accompanied by any regular alterations in the basal metabolic rate. Goldman *et al.* (1945) employed TP in the management of Graves' disease in 3 postmenopausal women, giving 25 mg. TP thrice weekly over a period of several months. In all 3, the Graves' disease was of considerable severity and all had an eventual reduction of the basal metabolic rate to normal levels and significant clinical improvement.

From the absence of a direct effect on the basal metabolic rate in the carefully controlled studies of Kinsell *et al.* (1944) and the difficulty in evaluating the factors contributing to a slow remission of symptoms in this disease, it is probable the effects of androgens are limited to the improvement of the metabolic status through nitrogen storage and the prevention of the loss of creatine from muscle, effects which TP achieves in a variety of debilitating states with protein depletion.

h. Muscular Dystrophy. The possible usefulness of androgens in this group of diseases derives from their effects in increasing muscle mass and function as well as the influence of TP in abolishing creatinuria, a defect characteristic of these diseases and directly related to the impaired muscle function. Additional support for the use of androgens rests on the predilection of this disease for the male, in whom its course is usually more acute than in the female, and its tendency to become less rapidly progressive after puberty. The report of therapeutic trials has been limited to that of Hoagland *et al.* (1944, 1945) who treated 5 children of both sexes between the ages of 7 and 12 with moderately advanced progressive muscular dystrophy. TP was given in daily amounts of 20 mg. for 10–14 days. In contrast with normal controls, no significant changes in urinary creatine output were observed. However, in both groups, the discontinuation of TP was followed by a marked increase in the excretion of urinary creatine and glycocyamine for a period of several days. This suggested that there may well have been a temporary storage of creatine in the muscles, and its subsequent discharge on discontinuation of hormonal therapy. Two patients were given MT for 10 days during which they exhibited a marked increase in urinary creatine and glycocyamine. Withdrawal of treatment with MT was followed by a rapid drop in the excretion of both urinary constituents, in contrast with the moderate rise after TP was discontinued. No effects on symptoms were reported and studies were still in progress on the effects of more prolonged treatment.

i. Debilitated States. It is beyond the scope of this review to do more than refer to the potential value of the protein anabolic as well as other metabolic effects of androgens which are now being extensively explored in a variety of debilitated states. The experimental background for this clinical application has been exhaustively reviewed by Kochakian (1946) and an evaluation of the status of this clinical application would be premature.

10. Miscellaneous

a. Diseases Characterized by Menstrual Exacerbations. A wide variety of conditions are prone to premenstrual and menstrual exacerbations. This has led to the attempt to influence these exacerbations by the use of androgens either premenstrually or throughout the intermenstrual interval, utilizing a wide variety of dosage levels. Beneficial results were stated to be achieved in tuberculosis (Pierre-Bourgeoise *et al.*, 1939; Chiray and Mollard, 1939), asthma (Chiray *et al.*, 1940), and migraine (Bühler, 1939). The results are difficult to evaluate on the basis of the published data. It would appear more logical to employ estrogens for premenstrual exacerbations of chronic diseases in view of the physiological reduction in estrogens during that phase of the menstrual cycle. However, such beneficial effects which androgens may exert in the disorders cited might be attributable to the relief of premenstrual tension in asthma and migraine and to their protein anabolic effects in chronic debilitating diseases.

b. Diseases with a High Incidence in Women. Androgens have also been used in several disorders characterized by an unusually high incidence in women, on the assumption that ovarian hormonal factors might be responsible for such preponderance in the female. Otosclerosis is much more common in women than men and its relation to the ovarian hormones is suggested by its frequent onset at puberty and exacerbation during pregnancy. Bernstien and Gillis (1939) employed androgens in the treatment of women with otosclerosis administering 5–10 mg. TP weekly for 12 weeks and thereafter at progressively longer intervals. Significant improvement was reported in 24 of 36 women, but the absence of audiograms deprives this observation of objective support. An improvement in hearing was observed in 12 of 24 women receiving TP in doses of 20 mg. a week (Prager, 1940). In a smaller series, this author alternated 10 mg. TP weekly with 2000 RU estradiol benzoate and noted improvement in 70%. Benefit from androgens has also been reported by Prietzel (1940). It is inferred that such benefit is due to a local increase in blood supply.

Lupus erythematosis has a three-fold higher incidence in women than in men. Rose and Pillsbury (1944) treated 8 patients with lupus erythematosis by means of 25 mg. TP daily with inconclusive results.

c. Disorders of the Skin. Scattered reports have appeared on the efficacy of androgens in the treatment of senile pruritus and pruritus vulvae. Feldman *et al.* (1942) failed to observe any benefit from androgens in senile pruritus except when given in combination with estrogens. Cinberg (1945) reports the relief of pruritus vulvae from the application of a bland ointment containing TP. The symptomatic relief was associated with the disappearance of evidence of inflammation immediately below the epidermal layer. A brief report on the use of androgens in eczema and psoriasis (Lafitte and Huret, 1939) contains too few details to permit evaluation. Despite the ample evidence that androgens produce acne, several reports have appeared on their use in the treatment of acne (Riley, 1939; Spilzinger, 1939, 1940). Those treated by Cornbleet and Barnes (1939) did poorly. Acne improved in 3 of 12 girls receiving 170–570 mg. TP over 3 months (Riley, 1939). All of Spilzinger's (1939) cases improved with doses of 60–120 mg. TP given over 2–4 weeks. The relation of the therapy to the improvement of the acne may be justifiably questioned.

d. Psychiatric Disorders. The literature is very meager relating to the use of androgens in psychiatric disorders. The most extensive study is that of Rothermich *et al.* (1941). They found androgens of no value in the management of classical involutional melancholia; however, 10 cases classified as atypical involutional psychosis associated with the menopause were regarded as responding favorably to TP in doses of 25–35 mg. thrice weekly for periods of 10–20 weeks. Any conclusions as to the specific relation of androgenic therapy to the improvement observed may be questioned in a condition prone to spontaneous remissions, particularly because of the long interval between the initiation of treatment and the onset of improvement.

Haas (1937) reported beneficial effects with androgens in 2 patients with menopausal depression of an unspecified character. A variety of psychotic states associated with excitation and with a large erotic component were given TP or TAc, the usual dose being 10 mg. for 7–20 days (Guiraud and Stora, 1939,a,b). A rapid sedative effect was observed with a diminution in erotic drive. In general the sparsity of reports and the inconclusive nature of the evidence permit no conclusions. The relationship of androgens to the psychological and emotional disturbances of the menopausal syndrome, which may be considered well established, has been discussed above.

V. Conclusions

Inasmuch as each clinical application of androgens has already been subjected to critical evaluation, it will suffice to conclude this review with a few comments of a general nature.

It is quite evident that the use of androgens in women has resulted in important contributions not only to our knowledge of the physiology of the reproductive system in the human, but also to the more effective management of a variety of clinical disorders. Further experience may be counted on to deal with unduly optimistic or unjustified evaluations of their therapeutic role. Although some clinical applications may prove only of present value, eventually to be superseded by measures which are more appropriate to, or specifically corrective of, the underlying defects, others may retain a permanent place in therapy by virtue of certain unique actions exerted by androgens. It has been possible to relate a number of their clinical effects to well established actions of androgens in women; on the other hand, there still remain many important areas of uncertainty, which await clarification.

It is also evident that, quite apart from their effects on reproductive physiology, the influence of androgens on the human organism is so widespread that it is no longer justifiable to regard them merely as male sex hormones, in the original narrow sense of the term. The mechanisms of many of their actions, such as those exerted on the cardiovascular and central nervous system, which are manifested by striking modifications in vasomotor, psychological and emotional behavior, have scarcely been explored. The property of androgens in inducing profound alterations in a variety of important metabolic functions is, however, receiving much attention not only for its scientific interest but also because of its therapeutic implications.

Finally, it would be unfair to ignore the opinion which has been vigorously expressed that androgens have no legitimate place in the treatment of women because they are unphysiological for this sex

(Hamblen, 1941, 1942). This criticism, which overlooks the participation of androgens in the normal hormonal economy of women, stems in large measure from an understandable anxiety over the possible induction of permanent virilization through their unskillful use. However, such undesirable consequences need not follow, except under special circumstances, if these agents are intelligently employed and the necessary precautions observed for their prevention—reservations which apply to any potent therapeutic agent.

REFERENCES

1. Abarbanel, A. R. 1939. *Am. J. Obstet. Gynecol.* **38,** 1043.
2. Abarbanel, A. R. 1940a. *Endocrinology* **26,** 765.
3. Abarbanel, A. R. 1940b. *Am. J. Obstet. Gynecol.* **39,** 243.
4. Abarbanel, A. R. 1941. *Am. J. Obstet. Gynecol.* **42,** 110.
5. Abel, S. 1945. *Am. J. Obstet. Gynecol.* **49,** 327.
6. Adair, F. E., and Herrmann, J. B. 1946. *Ann. Surg.* **123,** 1023.
7. Albright, F. 1942–1943. *Harvey Lectures, Series* **38,** 123.
8. Albright, F., Bloomberg, E., and Smith, P. H. 1940. *Trans. Assoc. Am. Physicians* **55,** 298.
9. Albright, F., Parsons, W., and Bloomberg, E. 1941. *J. Clin. Endocrinol.* **1,** 375.
10. Albright, F., Reifenstein, E. C., Jr., and Forbes, A. P. 1946. *Macy Conference Metabol. Aspects Convalescence* **12,** 97.
11. Albright, F., Smith, P. H., and Richardson, A. M. 1941. *J. Am. Med. Assoc.* **116,** 2465.
12. Allen, E. 1927. *Carnegie Inst. Wash. Pub. No.* **380,** *Contrib. Embryol.* **19,** 1.
13. Anderson, E., Haymaker, W., and Joseph, M. 1938. *Endocrinology* **23,** 398.
14. Atkins, H. J. B. 1940. *Lancet* **2,** 411.
15. Audit, M. J. 1938. *Compt. rend. soc. franç. gynécol.* **8,** 27.
16. Bartelmez, G. W. 1937. *Physiol. Revs.* **17,** 28.
17. Beato, V. 1946. *Intern. Abstracts Surg.* **83,** 260.
18. Beatson, G. T. 1896a. *Lancet* **2,** 104
19. Beatson, G. T. 1896b. *Lancet* **2,** 162.
20. Beckers, W. 1945. *Urol.* and *Cutaneous Rev.* **49,** 397.
21. Béclère, C. 1938. *Bull. soc. gynécol. obstet.* **27,** 747.
22. Béclère C. 1939a. *Presse méd.* **47,** 1149.
23. Béclère, C. 1939b. *Compt. rend. soc. franç gynécol.* **9,** 32.
24. Béclère, C. 1939c. *Bull. soc. gynécol. obstet.* **28,** 308.
25. Béclère, C. 1941. *Presse méd.* **49,** 166.
26. Béclère, C. 1945? *Questions gynécologiques d'actualité* p. 34.
27. Beecham, C. T. 1943. *Am. J. Obstet. Gynecol.* **46,** 849.
28. Beilly, J. S., and Solomon, S. 1940. *Endocrinology* **26,** 236.
29. Bender, X. 1937. *Compt. rend. soc. franç gynéc.* **7,** 222.
30. Benedek, T., and Rubenstein, B. B. 1939a. *Psychosomat. Med.* **1,** 245.
31. Benedek, T., and Rubenstein, B. B. 1939b. *Psychosomat. Med.* **1,** 461.
32. Berlind, M. 1941. *J. Clin. Endocrinol.* **1,** 986.
33. Bernstien, J., and Gillis, L. 1939. *Lancet* **2,** 1368.
34. Bickers, W. 1941. *Am. J. Obstet. Gynecol.* **42,** 1023.
35. Bickers, W 1942. *Virginia Med. Monthly* **69,** 423.

36. Birnberg, C. H., Kurzrok, L., and Klor, S. J. 1940. *Am. J. Obstet. Gynecol.* **39,** 107.
37. Birnberg, C. H., Kurzrok, L., and Livingston, S. 1938. *Endocrinology* **23,** 243.
38. Bishop, B. W. F. 1939. *S. African Med. J.* **13,** 211.
39. Biskind, G. R., Escamilla, R. F., and Lisser, H. 1941. *J. Clin. Endocrinol.* **1,** 38.
40. Black, E. F. E. 1942. *Can. Med. Assoc. J.* **47,** 124.
41. Bolger, W. P. 1946. *J. Clin. Endocrinol.* **6,** 88.
42. Bühler, F. 1939. *Deut. med. Wochschr.* **65,** 1739.
43. Burcura, C. J. 1910. *Zentr. Gynäkol.* **34,** 561.
44. Byron, C. S., and Katzen, P. 1941. *J. Clin. Endocrinol.* **1,** 359.
45. Callow, N. H., Callow, R. K., and Emmens, C. W. 1939. *J. Endocrinol.* **1,** 99.
46. Carter, A. C., and Shorr, E. Unpublished data.
47. Child, G. P., Woodbury, R. A., Torpin, R., Watson, W. G., and Jarboe, L. 1946. *Federation Proc.* **5,** 171.
48. Chiray, M., and Mollard, H. 1939. *Presse méd.* **47,** 681.
49. Chiray, M., Mollard, H., and Duret, M. 1940. *Presse méd.* **48,** 201.
50. Cinberg, B. L. 1942. *N. Y. State J. Med.* **42,** 2138.
51. Cinberg, B. L. 1945. *Am. J. Obstet. Gynecol.* **49,** 647.
52. Clahr, J. 1941. *J. Clin. Endocrinol.* **1,** 759.
53. Coggi, C. 1939. *Ann. ostet. ginecol.* **61,** 683.
54. Cornbleet, T., and Barnes, B. 1939. *Arch. Dermatol. and Syphilol.* **40,** 249.
55. Corner, G. W. 1927. *J. Am. Med. Assoc.* **89,** 1838.
56. Cowie, A. T., and Folley, S. J. 1946. *J. Endocrinol.* **4,** 375.
57. Daron, G. L. 1936. *Am. J. Anat.* **58,** 349.
58. Deakins, M. L., Friedgood, H. B., and Ferrebee, J. W. 1944. *J. Clin. Endocrinol.* **4,** 376.
59. de Allende, I. L. C., Shorr, E., and Hartman, C. G. 1943. *Carnegie Inst. Wash. Pub. No.* 557, *Contrib. Embryol.* **31,** 1.
60. Deanesly, R., and Parkes, A. S. 1937. *Proc. Roy. Soc., London B* **124,** 279.
61. del Castillo, E. B., and Salaber, J. A. 1939. *Semana méd.* **1,** 770.
62. Desmarest. 1938. *Semaine hôp. Paris* **14,** 251.
63. Desmarest and Capitain. 1937a. *Presse méd.* **45,** 1109.
64. Desmarest and Capitain. 1937b. *Presse méd.* **45,** 777.
65. Desmarest and Capitain. 1938. *Presse méd.* **46,** 185.
66. Desmarest and Capitain. 1939. *Presse méd.* **47,** 1031.
67. Di Paola, G., and del Castillo, E. B. 1942. *J. Clin. Endocrinol.* **2,** 215.
68. Dobriner, K. Personal communication.
69. Dobriner, K., and Shorr, E. 1946. Unpublished data.
70. Dorfman, R. I., and Hamilton, J. B. 1939. *Endocrinology* **25,** 33.
71. Douay, E. 1938. *Compt. rend. soc. franç. gynécol.* **8,** 165.
72. Douay, E. 1945? *Questions gynécologiques d'actualité* p. 30.
73. Duckman, S., and Turino, T. R. 1942. *Am. J. Obstet. Gynecol.* **44,** 112.
74. Duffy, P. V., and Corsaro, J. 1941. *J. Am. Med. Assoc.* **116,** 33.
75. Edwards, E. A., Hamilton, J. B., and Duntley, S. Q. 1939. *New Engl. J. Med.* **220,** 865.
76. Edwards, E. A., Hamilton, J. B., Duntley, S. Q., and Hubert, G. R. 1941. *Endocrinology* **28,** 119.
77. Emmens, C. W. 1941. *Endocrinology* **28,** 633.

78. Escamilla, R. F., and Lisser, H. 1941. *J. Clin. Endocrinol.* **1,** 633.
79. Evelbauer, K. 1939. *Münch. med. Wochschr.* **86,** 128.
80. Fabre, M. 1939. *Compt. rend. soc. franç. gynécol.* **9,** 211.
81. Farrow, J. H. 1944. *Surgery* **16,** 141.
82. Farrow, J. H., and Woodard, H. Q. 1942. *J. Am. Med. Assoc.* **118,** 339.
83. Feiman, D. T. 1941. *Ohio State Med. J.* **37,** 633.
84. Feldman, S., Pollock, J., and Abarbanel, A. R. 1942. *Arch. Dermatol. and Syphilol.* **46,** 112.
85. Fels, E. 1944. *J. Clin. Endocrinol.* **4,** 121.
86. Fevold, H. L. 1944. The Chemistry and Physiology of Hormones. Publication of the Am. Assoc. Advancement Sci. 152.
87. Fleischer, A. J., and Kushner, J. I. 1941. *J. Clin. Endocrinol.* **1,** 407.
88. Folley, S. J., Guthkelch, A. N., and Zuckerman, S. 1939. *Proc. Roy. Soc., London* **B126,** 469.
89. Folley, S. J., and Kon, S. K. 1938. *Proc. Roy. Soc., London* **B124,** 476.
90. Foote, F. W., and Stewart, F. W. 1945a. *Ann. Surg.* **121,** 6.
91. Foote, F. W., and Stewart, F. W. 1945b. *Ann. Surg.* **121,** 197.
92. Forbes, T. R. 1941. *Endocrinology* **29,** 70.
93. Foss, G. L. 1938. *Lancet* **1,** 992.
94. Frank, R. T. 1931. *Arch. Neurol. Psychiat.* **26,** 1053.
95. Freed, S. C. 1945. *J. Am. Med. Assoc.* **127,** 377.
96. Freed, S. C. 1946. *J. Clin. Endocrinol.* **6,** 571.
97. Gaines, J. A., Salmon, U. J., and Geist, S. H. 1938. *Proc. Soc. Exptl. Biol. Med.* **38,** 779.
98. Geist, S. H. 1941. *J. Clin. Endocrinol.* **1,** 154.
99. Geist, S. H., and Salmon, U. J. 1941. *J. Am. Med. Assoc.* **117,** 2207.
100. Geist, S. H., Gaines, J. A., and Salmon, U. J. 1940a. *Proc. Soc. Exptl. Biol. Med.* **44,** 319.
101. Geist, S. H., Salmon, U. J., and Walter, R. I. 1940b. *J. Am. Med. Assoc.* **114,** 1539.
102. Geist, S. H., Mintz, M., and Salmon, U. J. 1939. *Proc. Soc. Exptl. Biol. Med.* **41,** 556.
103. Geist, S. H., and Salmon, U. J. 1943. *J. Mt. Sinai Hosp.* **10,** 208.
104. Geist, S. H., Salmon, U. J., and Gaines, J. A. 1938a. *Endocrinology* **23,** 784.
105. Geist, S. H., Salmon, U. J., and Mintz, M. 1938b. *Am. J. Obstet. Gynecol.* **36,** 67.
106. Geschickter, C. F. 1943. Diseases of the Breast. Diagnosis, Pathology, Treatment. Lippincott, Philadelphia.
107. Goldman, A., Schaffer, A. I., and Markham, M. J. 1945. *N. Y. State J. Med.* **45,** 190.
108. Goldman, J. L., and Salmon, U. J. 1942. *Ann. Otol. Rhinol. Laryngol.* **51,** 961.
109. Greenblatt, R. B. 1940. *J. Am. Med. Assoc.* **115,** 120.
110. Greenblatt, R. B. 1942a. *J. Clin. Endocrinol.* **2,** 321.
111. Greenblatt, R. B. 1942b. *J. Med. Assoc. Georgia* **3,** 381.
112. Greenblatt, R. B. 1942c. *J. Clin. Endocrinol.* **2,** 665.
113. Greenblatt, R. B. 1943a. *J. Am. Med. Assoc.* **121,** 17.
114. Greenblatt, R. B. 1943b. *Am. J. Obstet. Gynecol.* **45,** 299.
115. Greenblatt, R. B., and Hair, L. Q. 1942. *J. Clin. Endocrinol.* **2,** 315.

116. Greenblatt, R. B., Mortara, F., and Torpin, R. 1942. *Am. J. Obstet. Gynecol.* **44,** 658.
117. Greenblatt, R. B., and Wilcox, E. A. 1941. *Southern Surgeon* **10,** 339.
118. Greene, R. R., Burrill, M. W., Oppenheimer, E., and Nelson, D. 1942. *Endocrinology* **30,** 734.
119. Greenhill, J. P., and Freed, S. C. 1939. *Western J. Surg. Obstet. Gynecol.* **47,** 301.
120. Greenhill, J. P., and Freed, S. C. 1940. *Am. J. Obstet. Gynecol.* **39,** 636.
121. Greenhill, J. P., and Freed, S. C. 1941. *J. Am. Med. Assoc.* **117,** 504.
122. Groome, J. R. 1939. *Lancet* **2,** 722.
123. Guiraud, P., and Stora, R. 1939a. *Ann. med. psychol.* **97,** 464.
124. Guiraud, P., and Stora, R. 1939b. *Presse méd.* **47,** 867.
125. Gusberg, S. B. 1945. *Am. J. Obstet. Gynecol.* **50,** 502.
126. Haas, A. 1937. *Fortschr. Therap.* **13,** 676.
127. Hamblen, E. C. 1941. *J. Clin. Endocrinol.* **1,** 180.
128. Hamblen, E. C. 1942. *J. Clin. Endocrinol.* **2,** 575.
129. Hamblen, E. C., Cuyler, W. K., and Axelson, G. J. 1941. *Endocrinology* **28,** 70.
130. Hamilton, J. B. 1941. *J. Clin. Endocrinol.* **1,** 570.
131. Hartman, C. G. 1932. *Carnegie Inst. Wash. Pub. No.* **433,** *Contrib. Embryol.* **23,** 1.
132. Hartman, C. G. 1937. *Proc. Soc. Exptl. Biol. Med.* **37,** 87.
133. Hartman, C. G. 1945. *J. Clin. Endocrinol.* **5,** 99.
134. Hartman, C. G., and Firor, W. 1937. *Quart. Rev. Biol.* **12,** 85.
135. Heller, C. G., Chandler, R. E., and Myers, G. B. 1944. *J. Clin. Endocrinol.* **4,** 109.
136. Heller, C. G., and Maddock, W. O. 1947. *Vitamins & Hormones* **5,** 393.
137. Hellman, A. M., and Ciner, L. F. 1941. *N. Y. State J. Med.* **41,** 30.
138. Henry, G. W., and Shorr, E. 1941. *U. S. Pub. Health Service, Pub. Health Rept., Suppl. No.* **168,** 125.
139. Herrmann, J. B., Adair, F. E., and Woodard, H. Q. 1947. *Surgery* **22,** 101.
140. Hirst, J. C. 1943. *Am. J. Obstet. Gynecol.* **46,** 97.
141. Hoagland, C. L., Gilder, H., and Shank, R. E. 1945. *J. Exptl. Med.* **81,** 423.
142. Hoagland, C. L., Shank, R. E., and Gilder, H. 1944. *Proc. Soc. Exptl. Biol. Med.* **55,** 49.
143. Hoffman, J. 1944. Female Endocrinology. Saunders, Philadelphia.
144. Hoskins, W. H., Coffman, J. R., Koch, F. C., and Kenyon, A. T. 1939. *Endocrinology* **24,** 702.
145. Howard, J. E., and Jewett, H. J. 1942. *J. Clin. Endocrinol.* **2,** 107.
146. Huffman, J. W. 1940. *Am. J. Obstet. Gynecol.* **40,** 675.
147. Huffman, J. W., and Bos, L. H. 1940. *Endocrinology* **26,** 259.
148. Huggins, R. A., and Woodbury, R. A. 1946. *Federation Proc.* **5,** 183.
149. Israel, S. L. 1938. *J. Am. Med. Assoc.* **110,** 1721.
150. Jacoby, A., and Rabbiner, B. 1943. *Am. J. Obstet. Gynecol.* **45,** 697.
151. Jeppson, E. M., Kasabach, H. Y., and Kantner, A. E. 1942. *J. Clin. Endocrinol.* **2,** 16.
152. Joël, C. A. 1942. *J. Clin. Endocrinol.* **2,** 116.
153. Jones, H. W. 1938. *Am. J. Obstet. Gynecol.* **35,** 64.
154. Keene, F. E., and Kimbrough, R. A. 1930. *J. Am. Med. Assoc.* **95,** 1164.
155. Kenyon, A. T., Sandiford, I., Bryan, A. H., Knowlton, K., and Koch, F. C. 1938. *Endocrinology* **23,** 135.
156. Kinsell, L. W., Hertz, S., and Reifenstein, E. C., Jr. 1944. *J. Clin. Invest.* **23,** 880.

157. Kinsell, L. W., Reifenstein, E. C. Jr., Bryant, D., and Albright, F. 1946. *J. Clin. Endocrinol.* **6**, 463.
158. Klopp, C., Young, N. F., and Taylor, H. C., Jr. 1945. *J. Clin. Invest.* **24**, 189.
159. Kochakian, C. D. 1938. *Endocrinology* **22**, 181.
160. Kochakian, C. D. 1942. *Macy Conference Metabol. Aspects Convalescence* **2**, 25.
161. Kochakian, C. D. 1946. *Vitamins & Hormones* **4**, 255.
162. Kochakian, C. D., and Murlin, J. R. 1935. *J. Nutrition* **10**, 437.
163. Kurzrok, L., Birnberg, C. H., and Livingston, S. 1939. *Endocrinology* **24**, 347.
164. Kurzrok, L., and Rothbart, H. 1942. *Am. J. Surg.* **56**, 636.
165. Kurzrok, R., and O'Connell, C. P. 1938. *Endocrinology* **23**, 476.
166. Kuschner, J. I. 1942. *J. Clin. Endocrinol.* **2**, 345.
167. Lacassagne, A. 1932. *Compt. rend.* **195**, 630.
168. Lacassagne, A., and Raynaud, A. 1939. *Compt. rend. soc. biol.* **131**, 586.
169. Lackner, J. E., Krohn, L., and Soskin, S. 1937. *Am. J. Obstet. Gynecol.* **34**, 248.
170. Lafitte, A., and Huret, G. 1939. *Presse méd.* **47**, 472.
171. Laroche, G., Simonnet, H., and Bompard, E. 1938. *Compt. rend. soc. biol.* **129**, 953.
172. Laroche, G., Simonnet, H., and Bompard, E. 1939. *Compt. rend. soc. biol.* **130**, 521.
173. Lass, P. M. 1942. *Am. J. Obstet. Gynecol.* **43**, 86.
174. Lattimer, J. K. 1942. *J. Urol.* **48**, 778.
175. Leff, W. A. 1941. *J. Clin. Endocrinol.* **1**, 562.
176. Lewis, D., and Geschickter, C. F. 1934. *Am. J. Surg.* **24**, 280.
177. Lipschütz, A., Vargas, L., and Ruz, O. 1939. *Lancet* **2**, 867.
178. Lisser, H., Escamilla, R. F., and Curtis, L. E. 1942. *J. Clin. Endocrinol.* **2**, 351.
179. Loeser, A. A. 1938a. *Lancet* **1**, 373.
180. Loeser, A. A. 1938b. *Lancet* **1**, 1134.
181. Loeser, A. A. 1939a. *Compt. rend. soc. franc. gynéc.* **9**, 77.
182. Loeser, A. A. 1939b. *Acta Union Intern. Contre Cancer* **4**, 375.
183. Loeser, A. A. 1940. *Brit. Med. J.* **1**, 479.
184. Loeser, A. A. 1941. *Lancet* **2**, 698.
185. Long, C. N. H., Katzin, B., and Fry, E. G. 1940. *Endocrinology* **26**, 309.
186. McCullagh, E. P. 1940. *Cleveland Clin. Quart.* **7**, 226.
187. McCullagh, E. P., and Rossmiller, H. R. 1941. *J. Clin. Endocrinol.* **1**, 496.
188. Mandy, T. E., and Mandy, A. J. 1940. *Western J. Surg. Obstet. Gynecol.* **48**, 604.
189. Marañón, G. 1938. *Lisboa méd.* **15**, 685.
190. Margiel, E., and Zwilling, E. 1936. *Polska gaz. lek.* **15**, 815.
191. Margolese, M. S. 1944. *J. Clin. Endocrinol.* **4**, 394.
192. Markee, J. E. 1940. *Carnegie Inst. Wash. Pub. No. 518, Contrib. Embryol.* **28**, 219.
193. Markee, J. E., and Berg, B. 1944. *Stanford Med. Bull.* **2**, 55.
194. Mazer, C., and Mazer, M. 1939. *Endocrinology* **24**, 599.
195. Mazer, C., and Mazer, M. 1940. *Penna. Med. J.* **43**, 1713.
196. Menkin, V. 1943. *Science* **97**, 165.
197. Meyer, R. 1930. *Handbuch der Gynäkologie München.* **6**, 211.

198. Miescher, K., Kägi, H., Scholz, C., Wettstein, A., and Tschopp, E. 1937. *Biochem. Z.* **294,** 39.
199. Miescher, K., and Tschopp, E. 1938. *Schweiz. med. Wochschr.* **62,** 1258.
200. Miescher, K., Wettstein, A., and Tschopp, E. 1936. *Biochem. J.* **30,** 1977.
201. Miller, J. R. 1944. *J. Am. Med. Assoc.* **125,** 207.
202. Mocquot, P., and Moricard, R. 1936a. *Bull. soc. obstet. gynécol.* **25,** 787.
203. Mocquot, P., and Moricard, R. 1936b. *Bull. soc. obstet. gynécol.* **25,** 791.
204. Moir, C. 1933–1934. *Trans. Edinburgh Obstet. Soc.* 93; in *Edinburgh Med. J.* Aug., 1934.
205. Muellner, S. R., and Hamilton, J. B. 1944. *J. Urol.* **52,** 139.
206. Nathanson, I. T. 1944. *Surgery* **16,** 108.
207. Nathanson, I. T. 1946a. *New Engl. J. Med.* **235,** 516.
208. Nathanson, I. T. 1946b. *New Engl. J. Med.* **235,** 548.
209. Nathanson, I. T., and Andervont, H. B. 1939. *Proc. Soc. Exptl. Biol. Med.* **40,** 421.
210. Nathanson, I. T., Meigs, J. V., and Parsons, L. 1942. *New Engl. J. Med.* **226,** 323; **226,** 487.
211. Nathanson, I. T., and Towne, L. E. 1939. *Endocrinology* **25,** 754.
212. Nelson, W. O. 1936. *Physiol. Revs.* **16,** 488.
213. Novak, E. 1926. *Am. J. Obstet. Gynecol.* **12,** 484.
214. Novak, E. 1939. *Am. J. Obstet. Gynecol.* **37,** 605.
215. Novak, E. 1941. Gynecology and Female Endocrinology. Little, Brown, Boston.
216. Novak, E., and Reynolds, S. R. M. 1932. *J. Am. Med. Assoc.* **99,** 1466.
217. Okkels, H., and Engle, E. T. 1938. *Acta Path. Microbiol. Scand.* **15,** 150.
218. Palmer, H. D., and DeRonde, M. 1943. *J. Clin. Endocrinol.* **3,** 428.
219. Palmer, R., and Moricard, R. 1938. *Compt. rend. soc. franç. gynécol.* **8,** 300.
220. Papanicolaou, G. N. 1933. *Am. J. Anat. Suppl.* **52,** 519.
221. Papanicolaou, G. N., and Falk, E. A. 1938. *Science* **87,** 238.
222. Papanicolaou, G. N., Ripley, H. S., and Shorr, E. 1938. *Proc. Soc. Exptl. Biol. Med.* **37,** 689.
223. Papanicolaou, G. N., Ripley, H. S., and Shorr, E. 1939. *Endocrinology* **24,** 339.
224. Papanicolaou, G. N., and Shorr, E. 1936. *Am. J. Obstet. Gynecol.* **31,** 806.
225. Peralta Ramos, A., and Colombo, E. O. 1938. *Bol. acad. nac. med. Buenos Aires* 429.
226. Perloff, W. H. 1942. *J. Clin. Endocrinol.* **2,** 419.
227. Perloff, W. H., Rose, E., and Sunderman, F. W. 1943. *Arch. Internal Med.* **72,** 494.
228. Pierre-Bourgeois, and Boquet-Jesensky, M. 1939. *Presse méd.* **47,** 511.
229. Pollano, O. 1924. *Z. Geburtsh. Gynäkol.* **87,** 363.
230. Portes, L., Dalsace, J., and Wallich, R. 1939. *Compt. rend. soc. biol.* **130,** 1100.
231. Prager, J. B. 1940. *Med. Record* **152,** 261.
232. Preissecker, E. 1940. *Zentr. Gynäkol.* **64,** 999.
233. Prietzel, F. 1940. *Monatschr. Ohrenh.* **74,** 18.
234. Prudente, A. 1945. *Surg. Gynecol. Obstet.* **80,** 575.
235. Reifenstein, E. C. Jr., Forbes, A. P., Albright, F., Donaldson, E., and Carroll, E. 1945. *J. Clin. Invest.* **24,** 416.
236. Reynolds, S. R. M., Hamilton, J. B., DiPalma, J. R., Hubert, G. R., and Foster, F. I. 1942. *J. Clin. Endocrinol.* **2,** 228.

237. Reynolds, S. R. M., Kaminester, S., and Schloss, S. 1940. *Proc. Soc. Exptl. Biol. Med.* **45**, 749.

238. Riley, I. D. 1939. *Brit. J. Dermatol. Syphilis* **51**, 119.

239. Robson, J. M. 1937a. *J. Physiol.* **90**, 15p.

240. Robson, J. M. 1937b. *Quart. J. Exptl. Physiol.* **26**, 355.

241. Robson, J. M. 1937c. *Proc. Soc. Exptl. Biol. Med.* **36**, 153.

242. Rose, E., and Pillsbury, D. M. 1944. *Ann. Internal Med.* **21**, 1022.

243. Rosenburg, A. 1922. *Frankfurt. Z. Path.* **27**, 466.

244. Rothermich, N. O. 1939. *Endocrinology* **25**, 520.

245. Rothermich, N. O., and Foltz, L. M. 1940. *Endocrinology* **27**, 37.

246. Rothermich, N. O., Postle, B., and Foltz, L. M. 1941. *Arch. Neurol. Psychiat.* **45**, 752.

247. Rubenstein, H. S. 1939. *J. Am. Med. Assoc.* **112**, 537.

248. Rubenstein, H. S., and Abarbanel, A. R. 1939. *Am. J. Obstet. Gynecol.* **37**, 709.

249. Rubenstein, H. S., Shapiro, H. D., and Freeman, W. 1940. *Am. J. Psychiat.* **97**, 703.

250. Rutherford, R. N. 1942. *Western J. Surg. Obstet. Gynecol.* **50**, 282.

251. Salaber, J. A., and del Castillo, E. B. 1942. *Medicine, Buenos Aires* **2**, 483.

252. Salaber, J. A., Sardi, J. L., and Arrighi, L. 1943. *Bol. soc. obstet. ginecol. Buenos Aires* **22**, 301.

253. Salmon, U. J. 1937. *Proc. Soc. Exptl. Biol. Med.* **37**, 488.

254. Salmon, U. J. 1941. *J. Clin. Endocrinol.* **1**, 162.

255. Salmon, U. J., and Geist, S. H. 1943. *J. Clin. Endocrinol.* **3**, 235.

256. Salmon, U. J., Geist, S. H., Gaines, J. A., and Walter, R. I. 1941a. *Am. J. Obstet. Gynecol.* **41**, 991.

257. Salmon, U. J., Geist, S. H., and Salmon, A. A. 1941b. *Proc. Soc. Exptl. Biol. Med.* **48**, 11.

258. Salmon, U. J., Geist, S. H., and Walter, R. I. 1939. *Am. J. Obstet. Gynecol.* **38**, 264

259. Sampson, J. A. 1927. *Am. J. Obstet. Gynecol.* **14**, 422.

260. Schrire, I., and Sharpey-Schafer, E. P. 1938. *Clin. Sci.* **3**, 412.

261. Schröder, R. 1928. *Am. J. Obstet. Gynecol.* **16**, 155.

262. Seckinger, D. L. 1923. *Bull. Johns Hopkins Hosp.* **34**, 236.

263. Seckinger, D. L., and Corner, G. W. 1923. *Anat. Record* **26**, 299.

264. Selye, H. 1941. *J. Endocrinol.* **2**, 352.

265. Severinghaus, A. E. 1939. Sex and Internal Secretions. 2nd ed., edited by E. Allen. Williams & Wilkins, Baltimore. 1045.

266. Sevringhaus, E. L., and Sikkema, S. 1946. *J. Clin. Endocrinol.* **6**, 415.

267. Sharpey-Schafer, E. P. 1940. *Lancet* **1**, 161.

268. Shimkin, M. P., Lorenz, E., Wyman, R., and Norton, S. G. 1944. *Endocrinology* **35**, 283

269. Shorr, E. 1940a. *Proc. Soc. Exptl. Biol. Med.* **43**, 501

270. Shorr, E. 1940b. *Bull. N. Y. Acad. Med.* **16**, 453.

271. Shorr, E. 1942. *Macy Conference Metabol. Aspects Convalescence* **2**, 12.

272. Shorr, E. 1945. *J. Mt. Sinai Hosp.* **12**, 667.

273. Shorr, E. Unpublished data.

274. Shorr, E. and Carter, A. C. Unpublished data.

275. Shorr, E., and Cohen, E. J. 1940. *Proc. Soc. Exptl. Biol. Med.* **46**, 330.

276. Shorr, E., Hotchkiss, R. S., and Harrison, H. O. Unpublished data.

277. Shorr, E., Papanicolaou, G. N., and Stimmel, B. F. 1938. *Proc. Soc. Exptl. Biol. Med.* **38,** 759.
278. Shute, E. 1941. . *Am. J. Obstet. Gynecol.* **42,** 490.
279. Siegler, S. L., and Silverstein, L. M. 1940. *Am. J. Obstet. Gynecol.* **39,** 109
280. Silberman, D., Radman, H. M., and Abarbanel, A. R. 1940. *Am. J. Obstet. Gynecol.* **39,** 332.
281. Simon, A. F. 1946. *N. Y. State J. Med.* **46,** 743.
282. Smith, O. W., and Smith, G. Van S. 1946. *J. Clin. Endocrinol.* **6,** 483.
283. Speert, H. 1941. *Surg., Gynecol. Obstet.* **73,** 388.
284. Speert, H. 1948. *Carnegie Inst. Wash. Pub. Contrib. Embryol.* **32,** in press.
285. Spence, A. W. 1939. *Lancet* **2,** 820.
286. Spence, A. W. 1940. *Lancet* **2,** 387.
287. Spilzinger, J. M. 1939. *Semana méd.* **2,** 939.
288. Spilzinger, J. M. 1940. *Rev. asoc. méd. argent.* **54,** 740.
289. Steinach, E., and Kun, H. 1937. *Lancet* **2,** 845.
290. Stewart, H. L., Jr., and Pratt, J. P. 1941. *Am. J. Obstet. Gynecol.* **41,** 555.
291. Stone, E. T. R. 1941. *Am. J. Obstet. Gynecol.* **42,** 114.
292. Sturgis, S. H., and Albright, F. 1940. *Endocrinology* **26,** 68.
293. Sturgis, W. A., Abarbanel, A. R., and Nadar, D. S. 1940. *Am. J. Obstet. Gynecol.* **39,** 102.
294. Sutton, L. A. 1940. Endometriosis. Davis' Gynecology and Obstetrics. W. F. Prior Co., Hagerstown, Md., Chap. 16.
295. Tailhefer, A. 1938. *Paris méd.* **109,** 189.
296. Talbot, N. B., Butler, A. M., and MacLachlan, E. A. 1943. *J. Clin. Invest.* **22,** 583.
297. Taylor, H. C., Jr. 1942. *Surg., Gynecol. Obstet.* **74,** 326.
298. Taylor, H. C., Jr., Mecke, F. E., and Twombly, G. H. 1943. *Cancer Research* **3,** 180.
299. Taylor, H. C., Jr., and Waltman, C. L. 1940. *Arch. Surg.* **40,** 733.
300. Thorn, G. W., and Engel, L. L. 1938. *J. Exptl. Med.* **68,** 299.
301. Traut, H. F., and Kuder, A. 1935. *Surg. Gynecol. Obstet.* **61,** 145.
302. Turner, C. W. 1939. Sex and Internal Secretions. 2nd ed., edited by E. Allen. Williams & Wilkins, Baltimore, 740.
303. Turpault, M. P. 1937. *Compt. rend. soc. franç gynécol.* **7,** 181.
304. Turpault, M. P. 1939. *Gynécologie* **38,** 281.
305. Turpault, M. P. 1945? *Questions gynécologiques d'actualité,* p. 40.
306. Ulrich, P. 1938. *Compt. rend. soc. franç. gynécol.* **8,** 278.
307. Ulrich, P. 1939a. *Compt. rend. soc. franç. gynécol.* **9,** 70.
308. Ulrich, P. 1939b. *Compt. rend. soc. franç. gynécol.* **9,** 75.
309. Ulrich, P. 1939c. *Acta Union Intern. Contre Cancer* **4,** 377.
310. Van Wagenen, G., and Folley, S. J. 1939a. *Anat. Record.* **73,** *Suppl.* **2,** 54.
311. Van Wagenen, G., and Folley, S. J. 1939b. *J. Endocrinol.* **1,** 367.
312. Vest, S. A., and Howard, J. E. 1939. *J. Am. Med. Assoc.* **113,** 1869.
313. von Konrád, E. 1939. *Zentr. Gynäkol.* **63,** 2429.
314. Walsh, J. W., and Stromme, W. B. 1944. *Am. J. Obstet. Gynecol.* **47,** 654.
315. Weil, P. G., and Browne, J. S. L. 1940. *J. Clin. Invest.* **19,** 772.
316. Werner, S. C., and West, R. 1943. *J. Clin. Invest.* **22,** 335.
317. White, A. 1943. *Ann. N. Y. Acad. Sci.* **43,** 341.
318. Wilkins, L., and Fleischmann, W. 1944. *J. Clin. Endocrinol.* **4,** 306.
319. Wilkins, L., and Fleischmann, W. 1945. *J. Clin. Invest.* **24,** 21.

320. Wilkins, L., and Fleischmann, W. 1946. *J. Clin. Endocrinol.* **6,** 383.
321. Wilkins, L., Fleischmann, W., and Howard, J. E. 1941. *Bull. Johns Hopkins Hosp.* **59,** 493.
322. Williams, G. A. 1943. *Urol. Cutaneous Rev.* **47,** 554.
323. Williams, R. H., and Whittenberger, J. L. 1942. *J. Clin. Endocrinol.* **2,** 539.
324. Williams, R. H., Whittenberger, J. L., Bissell, G. W., and Weinglass, A. R. 1945. *J. Clin. Endocrinol.* **5,** 163.
325. Wilson, L. 1940. *Endocrinology* **27,** 29.
326. Wilson, L., and Kurzrok, R. 1938. *Endocrinology* **23,** 79.
327. Wilson, L., and Kurzrok, R. 1940a. *Endocrinology* **26,** 587.
328. Wilson, L., and Kurzrok, R. 1940b. *Endocrinology* **27,** 23.
329. Wollner, A. 1941. *J. Clin. Endocrinol.* **1,** 228.
330. Woodbury, R. A., Child, G. P., Torpin, R., Watson, W., G. and Jarboe, L. 1946. *Federation Proc.* **5,** 215.
331. Woodbury, R. A., Torpin, R., Child, G. P., Watson, H., and Jarboe, M. 1947. *J. Am. Med. Assoc.* **134,** 1081.
332. Wyatt, J. 1945. *J. Obstet. Gynecol. Brit. Empire* **52,** 174.
333. Zuckerman, S. 1937. *Lancet* **2,** 676.
334. Zuckerman, S. 1941. *J. Endocrinol.* **2,** 263.

The Clinical Uses of Testosterone in the Male*

By CARL G. HELLER AND WILLIAM O. MADDOCK

CONTENTS

Page
I. Introduction 394
II. Hypogonadism 394
  1. Evaluation of Laboratory Determinations that Might Aid in Distinguishing Hypogonadism from Normal. 395
    a. Urinary Androgen Excretion. 395
    b. Urinary Estrogenic Activity 396
    c. Urinary 17-Ketosteroids. 396
    d. Urinary Gonadotropins. 397
    e. Testicular Biopsies. 398
  2. Functional Pathology of Hypogonadism. 398
    a. Normal Mechanisms 398
    b. Functional Pathology. 400
  3. Clinical Features. 400
    a. Secondary or Hypogonadotropic Hypogonadism 400
    b. Primary or Hypergonadotropic Hypogonadism. 404
  4. Details of the Hypergonadotropic Syndromes 406
    a. Hypergonadotropic Eunuchoidism 406
    b. Adult Hypergonadotropic Hypogonadism 408
    c. Puberal Seminiferous Tubule Failure 410
    d. Adult Seminiferous Tubule Failure. 411
III. Testosterone Administration 411
  1. Inunction 412
  2. Oral. 412
  3. Sublingual. 412
  4. Intramuscular or Subcutaneous Injection 413
  5. Pellet Implantation. 413
IV. Changes Effected by Androgen Administration 414
V. Effect of Exogenous Testosterone on Spermatogenesis 415
VI. Use of Testosterone in the Prepuberal Child 417
VII. Cryptorchidism. 417
VIII. Impotence 418
IX. Homosexuality 419
X. Angina Pectoris. 422
XI. Benign Prostatic Hypertrophy 425
XII. Conclusions. 427
XIII. Summary 427
References 428

---

* These studies have been supported in part by grants from the Schering Corporation, Bloomfield, N. J., Dr. Edward Henderson, Medical Director.

I. INTRODUCTION

The diagnosis and treatment of hypogonadism has recently been modified by the application of two important technics; the determination of urinary gonadotrophins and the histological examination of testicular biopsies. The application of these two technics yields important information concerning the nature of the primary disease process involved in individual patients and permits therapy to be instituted on the basis of correcting functional pathology. This is in contrast to much of the early work in which it was characteristic to note the results of administering testosterone to essentially undiagnosed cases having testicular insufficiency. No matter how objective the statistical treatment of such data or how many times these data are multiplied, no new information concerning the fundamental nature of the disease process or its functional pathology results. For this reason, much of the early work concerning the use of testosterone in hypogonadism has not been included in this review. For a summary of the early work concerning the therapeutic use of testosterone in hypogonadism, the reader is referred to the excellent reviews of the Council on Pharmacy and Chemistry (1939), Hamilton (1941), Howard (1941), Kenyon (1941), Pullen, Wilson *et al.* (1942a,b,c), Aub and Kety (1943) and Vest and Barelare (1943).

Testosterone has been reported to be beneficial in many diseases, neither associated with, nor related to, hypogonadism. It has been deemed necessary to devote considerable space to critical analysis of these reports, since they often have had influence far out of proportion to their merit.

II. HYPOGONADISM

What is "hypogonadism"? It is a descriptive term used to indicate a lack of gonadal function. "Hypogonadism" is not a definitive diagnosis in that the concept includes such pathological states as the lack of gonadal development encountered in panhypopituitarism (Simmond's disease). The definitive diagnosis in panhypopituitarism is applied to the pathological process involving the pituitary gland rather than to the resultant hypogonadism. "Hypogonadism" also includes such physiological states as the lack of gonadal development encountered in prepuberal children.

What is included in the term "hypogonadism"? The gonad has two functions: hormonal, the production of androgen; and reproductive, the production of spermatozoa. "Hypogonadism" includes lack of the hormonal function, or lack of the reproductive function, or lack of both functions. The eunuch and the prepuberal child serve as examples of hypogonadism in which both functions are lacking. Sterile adults retaining normal hormonal function may be considered hypogonadal if they lack the capacity to produce spermatazoa. Lack of hormonal function alone is never encountered because of the partial dependence of spermatogenesis upon secretion of androgen. However, the defect in certain hypogonadal individuals may involve primarily the hormonal function and spermatogenesis may be affected only secondarily.

How is it determined that hypogonadism exists? In some patients hypogonadism is apparent from the physical examination. In others very little information concerning the status of gonadal function is derived from the physical examination. It is especially difficult to detect hypogonadism in persons who develop gonadal failure after sexual maturity has been attained, because regressive changes in the secondary sex characteristics occur slowly, if at all. After castration, either functional or surgical, the size of the penis rarely recedes appreciably. The voice remains normal. The hair distribution remains approximately the same, although some regression of beard and bodily hair may be noted after many years. The skeleton, by virtue of prior epiphyseal closure, does not change. The testes themselves may exhibit no appreciable gross alteration in such instances of adult hypogonadism as the male climacteric. Therefore, the withdrawal symptoms common to adult gonadal failure are easily ascribed as being of psychoneurotic origin. Since in gonadal failure specific hormonal therapy is indicated, and in neuroses psychotherapy is indicated, it is important to make the distinction between them.

The inability to differentiate all cases of hypogonadism from normal on clinical grounds alone leads naturally to investigating the value of laboratory procedures as diagnostic aids. The potentially valuable procedures include determinations of the urinary sex hormones or their degradation products, urinary gonadotropins and examination of testicular biopsies.

1. Evaluation of Laboratory Determinations that Might Aid in Distinguishing Hypogonadism from Normal

a. Urinary androgen excretion might be expected to be significantly lower than normal in hypogonadal men. However, when one considers that the degradation products of steroid hormones derived from either the testis or adrenal cortex possess androgenic activity, then a deficiency of the testis might not be expected to lead to a marked drop in urinary androgen. In fact, Dingemanse, Borchardt, and Laqueur (1937) failed to find any essential difference in androgen excretion between men and women, owing, most likely, to the fact that the adrenal gland contributed a large share of the androgenic activity in the women. Gallagher, Peterson *et al.* (1937) found remarkably high androgen excretion values for women (42–56 international units) as contrasted to men (63–68 I.U.). It is hardly to be expected, therefore, that testicular failure would result in any significant drop. Kenyon, Gallagher *et al.* (1937) confirmed this prediction in assays of urines from eunuchoidal subjects. They found that androgen excretion averaged about $\frac{1}{3}$ of normal but that values for low normals were overlapped. Thus, this test cannot be relied upon to make a differentiation between hypogonadal and normal in individual cases.

b. Urinary estrogenic activity is a property of the degradation products of certain steroids derived from the adrenal cortex, ovary and testis. One could, therefore, predicate a fall in urinary estrogenic activity as a result of testicular failure and further predicate that the fall would not be striking. Gallagher *et al.* (1937) found from 2 to 29 γ equivalents of estrone per day in 4 normal individuals and 1–9 γ/day in 7 eunuchoids (Kenyon *et al.*, 1937). In each of two different eunuchoidal syndromes, depressed estrogen values were found (Heller, Nelson, and Roth, 1943; Heller and Nelson, 1945a) but overlapping with low normal levels was encountered in each series. Thus, estrogen assays will not clearly delineate hypogonadism from normal in individual cases.

c. Urinary 17-ketosteroids are those hormones or degradation products of steroid hormones of testicular, ovarian and adrenal cortical derivation that have a ketone group attached to carbon atom number 17 in the cyclopentanophenanthrene nucleus. The 17-ketosteroids react similarly to certain chemical manipulations, allowing a common color reaction to serve as the assay end-point. During the process, estrone, a 17-ketosteroid, is eliminated. It has been calculated that approximately $\frac{3}{8}$ of the total urinary 17-ketosteroids are derived from testicular secretions and approximately $\frac{5}{8}$ from adrenal cortical secretions (Fraser, Forbes *et al.*, 1941; Talbot and Butler, 1942; Scott and Vermeulen, 1942). These chemically related steroids are biologically heterogeneous, so that no relationship necessarily exists between the excretion of 17-ketosteroids in milligrams and the biological androgenic activity of the urine. Testosterone, for example, is not a 17-ketosteroid and aetiocholanolone, a 17-ketosteroid found abundantly in urine, is not an androgen.

In hypogonadism average values for urinary 17-ketosteroids are lower than average values for normal men (Callow, Callow, and Emmens, 1940; Fraser *et al.*, 1941; Klinefelter, Reifenstein, and Albright, 1942; Heller, Nelson, and Roth, 1943; and Heller and Nelson, 1945a). In each of these reported series of cases, serious overlapping between values obtained in single assays and for single individuals occurs between the normal and hypogonadal groups. This precludes the use of 17-ketosteroid determinations for making the distinction between normal and hypogonadal men in individual instances.

Since there has been a tendency on the part of some workers to attach diagnostic significance to minor changes in sex hormone and 17-ketosteroid excretion or to minor alterations in estrogen-androgen ratios (see page 419), and since the use of testosterone has been justified on the basis of these minor variations the following observations seem cogent: Callow and Callow (1940), discussing 17-ketosteroid excretion, state: "A high level, . . . , is the only observation with a definite and independent diagnostic value; it occurs in cases of adrenal cortical tumour." Werner (1943), after studying daily variations in estrogen and 17-ketosteroid output, states, "The estrogen-androgen ratio reveals a tremendous variation from day to day in the same person. Thus it does not appear to have any value in clinical practice." The data of Gallagher *et al.* (1937) conforms with this conclusion.

d. The urinary gonadotropins are protein hormones, presumably secreted by the anterior pituitary, and excreted in the urine as follicle-stimulating hormone (FSH) and interstitial cell-stimulating hormone (ICSH). These hormones are concentrated from the urine by a variety of methods and assayed in immature female rats or mice, the end-point being changes in ovarian and/or uterine weight. The relative value of different assay methods has been discussed by Thayer (1946) in the preceding volume. It has been demonstrated that by using each of several concentration methods, a clear-cut distinction can be made between urines containing normal amounts, higher than normal amounts and lower than normal amounts (Heller and Heller, 1939; Varney and Koch, 1942; Heller and Chandler, 1942; Klinefelter, Albright, and Griswold, 1943; Smith, Albright, and Dodge, 1943, using modifications of the alcohol precipitation method; Jungck, Maddock, and Heller, 1947, using ultrafiltration). Reference to the various methods using tannic acid as the precipitating agent are omitted here, since, as Werner (1943) points out, random samples from normal individuals using this method often reveal less than a determinable amount; thus, the distinction between normal excretion and hypoexcretion is made with great difficulty.

Gonadotropic values obtained from normal men vary as greatly as values for estrogen, androgen and 17-ketosteroid excretion among single assays and among single individuals (Heller, Heller, and Sevringhaus, 1941; Werner, 1943). In hypogonadism, gonadotropin titers may be either elevated or depressed, depending upon the primary cause of the hypogonadism. In those syndromes in which elevated titers have been found they have been unmistakably higher than normal. The hypogonadal syndromes in which there is an associated elevation in titer include: (1) pre- and postpuberal castration (Hamilton, Catchpole, and Hawk, 1945), (2) functional prepuberal castration (Heller, Nelson, and Roth, 1943), (3) male climacteric (Heller and Myers, 1944) and (4) hyalinization of the seminiferous tubules (Klinefelter, Reifenstein, and Albright, 1942; Heller and Nelson, 1945a). In those syndromes in which depressed titers have been found, they have been unmistakably lower than normal. The hypogonadal syndromes in which there is an associated decrease in titer of gonadotropins include (1) panhypopituitarism (Klinefelter *et al.*, 1943; Fraser *et al.*, 1941) and (2) hypogonadotropic eunuchoidism (Heller and Nelson, 1944). No proved cases of hypogonadism have been shown to have gonadotropin titers within the range of variation for normal men.

The diagnostic value of gonadotropin titers in hypogonadism is that the abnormal deviations are so great that no overlapping with the range for normal men occurs. Thus, in a given individual a positive conclusion can be drawn from two to four assays on as many urine samples, using the alcohol precipitation or ultrafiltration concentration methods.

e. Testicular biopsies are simply and easily obtained (Charny, 1940) and are of great significance in arriving at the conclusion that hypogonadism exists. They may also afford information as to the genesis of the hypogonadism. Testicular biopsies alone do not necessarily afford a sure means for concluding that hypogonadism is present since, as Charny and Meranze (1942), Sand and Okkels (1938) and Oberndorfer (1930) have shown, many systemic diseases may cause a morphological change in the testes. Following recovery, testis function may be normal but some morphological change may persist. On the other hand, all proved cases of hypogonadism in which testicular biopsies have been studied show definite testicular derangement (Klinefelter *et al.*, 1942; Heller, Nelson, and Roth, 1943; Heller and Nelson, 1946; Nelson and Heller, 1945).

Therefore, the most certain way to establish the existence of hypogonadism is to determine urinary gonadotropins and to examine testicular biopsy specimens.

What treatment should be chosen for hypogonadism? Treatment of hypogonadism will depend upon the genesis of the hypogonadism in a given instance. Before any discussion of treatment can be undertaken, the functional pathology of hypogonadism must be examined.

2. Functional Pathology of Hypogonadism

a. Normal Mechanisms. The pituitary gonadotropic hormones (FSH and ICSH), the testis, and the end-products of testicular function may be divided into two systems: germinal and hormonal. The germinal or reproductive system (Fig. 1—left side) is represented by the stimulus, follicle-stimulating hormone[1] (FSH), which stimulates the germinal element of the testis, seminiferous tubules, to produce the end-product spermatozoa. The hormonal system (Fig. 1—right side) is represented by the stimulus, interstitial cell-stimulating hormone[2] (ICSH), which acts upon the hormonal element of the testis, interstitial cells of Leydig, to produce the end-product androgen (presumably testosterone).

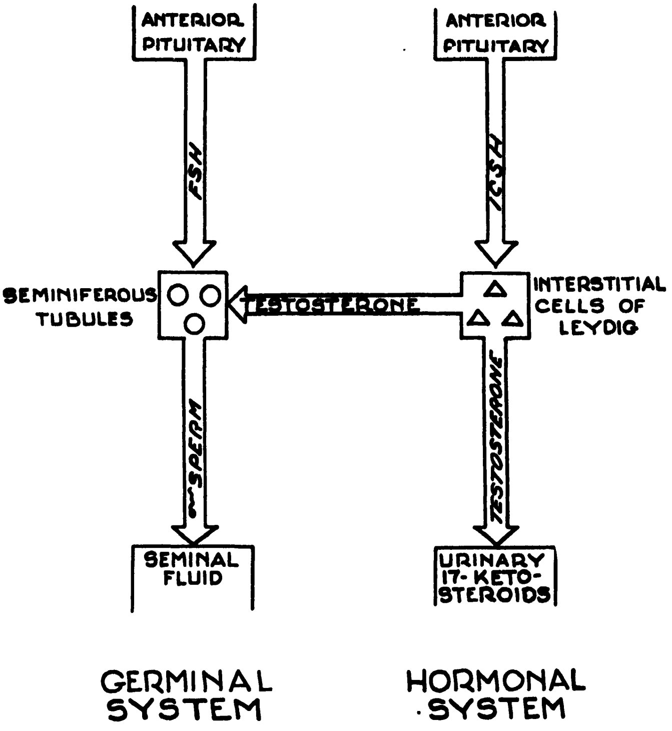


FIG. 1.—Diagram of the normal hormonal relationships involving the testis.

The two systems depicted in Fig. 1 form the two vertical limbs of the letter "H." They are functionally separate except for the partial dependence of the seminiferous tubules upon testosterone, which in the diagram forms the horizontal bar of the "H." Testosterone aids in maintaining the seminiferous tubules just as it maintains the genital tract

---

[1] So named because in the female it stimulates the morphological development of ovarian follicles.

[2] Also called luteinizing hormone (L. H.), which must be distinguished from the third gonadotropic hormone "luteotropic hormone." The latter has not as yet been ascribed a function in male reproductive physiology.

(epididymis, vas deferens, seminal vesicles, prostate and external genitalia) of which the seminiferous tubules are an integral part.

Finding normal amounts, motility and morphology of spermatozoa signifies that the entire "H" is intact. Finding defects in sperm production signifies an abnormality, which may involve any part of the system.

b. Functional Pathology. It can readily be appreciated from the normal physiology involved, that failure in certain portions of the "H" may lead to alterations in structure or function in other parts of the system. Failure of FSH secretion would lead to seminiferous tubule failure and sperm would not be produced. Failure of ICSH secretion would lead to Leydig cell failure, testosterone would not be secreted and it, in turn, would fail to maintain secondary sex characteristics and fail to maintain seminiferous tubule function. Clinical investigations have not as yet revealed instances in which FSH and ICSH have failed separately. Simultaneous failure of both stimulators of the testis is well known in panhypopituitarism where all trophic pituitary hormones fail to be secreted, and in hypogonadotropic hypogonadism, where both FSH and ICSH fail to be secreted. The resultant hypogonadism has been referred to as "secondary" and is invariably associated with a distinct decrease in urinary gonadotropin excretion.

Failure of Leydig cell secretion results in seminiferous tubule failure, thus resulting in lack of both the hormonal and germinal end-products of testicular function. Seminiferous tubule failure could result in lack of sperm only. Both types of failure have been noted clinically, although most commonly the disease or injury which involves one function of the testis does not spare the other. The resultant hypogonadism has been referred to as "primary" and is invariably associated with a distinct increase in urinary gonadotropin excretion.

The above is important in diagnosis because all instances of hypogonadism may be divided according to their gonadotropin titers into those in which testicular failure is "primary" in the testis (high titers) and those in which the testicular failure is "secondary" to pituitary failure (low titers).

It is similarly important in establishing the proper therapy because the instances of primary testicular failure (high gonadotropin titers) will in practice require *substitutional* therapy with testosterone and the instances of secondary testicular failure (low gonadotropin titers) will require *stimulatory* therapy with the gonadotropic hormones.

3. Clinical Features

a. In secondary or hypogonadotropic hypogonadism the clinical features will depend upon such accompanying circumstances as (1) time of onset, *i.e.*, pre- or postpuberal and (2) the functions of the anterior pituitary that are involved in failure, *i.e.*, all the trophic hormone or only the gonadotropic hormones. The combinations of these two circumstances permit the existence of four hypogonadotropic syndromes (Table I). These will include the following types of patients: dwarfs with infantilism, eunuchoids, cachectic hypogonadal individuals, normal-appearing hypogonadal individuals. Also included in this category is the normal child. They have in common equally low gonadotropin titers (Fraser *et al.*, 1941; Klinefelter *et al.*, 1943; Heller and Nelson, 1946) and histological evidence of unstimulated testes, in which both Leydig cells and germinal elements are found in an unstimulated state, not unlike that seen in hypophysectomized animals.

When the onset of the failure occurs before or at puberty, and continues to be present as the age of puberty and age of adulthood are attained, skeletal changes characterize the condition. When all the trophic hormones are involved, sexual infantilism is associated with dwarfism and often with signs of adrenal and thyroid failure. If only the gonadotropic hormones fail, the resultant infantilism is associated with eunuchoidism (Heller and Nelson, 1946).

When the onset of the failure occurs after sexual maturation has been attained, few clinically apparent evidences of hypogonadism are noted, often only sterility and impotence. When all the trophic hormones are involved, evidences of thyroid and/or adrenal cortical failure may be present and the diagnosis of panhypopituitarism (Simmond's cachexia) is made. When only the gonadotropic hormones fail, no thyroid, adrenal cortical or other target-organ failure is noted. The single example of this syndrome (Heller, unpublished) had climacteric symptoms and

TABLE I

Hypogonadotropic Hypogonadism

Type of pituitary deficiency	Time of onset	Condition
All trophic factors deficient	Before puberty	Panhypopituitary dwarfism
	After maturation	Simmond's cachexia
Only gonadotropic factor deficient	Before puberty	Normal child
	Continued through puberty into adult life	Hypogonadotropic eunuchoidism
	After maturation	Adult hypogonadotropic hypogonadism

Whereas all of the above conditions will respond to testosterone administration, gonadotropin administration is more physiological and potentially less harmful. (The normal child, of course, requires neither form of therapy.)

impotence as the only complaints. The four hypogonadotropic syndromes are summarized in Table I. It is to be noted that lack of gonadotropin secretion (but not of the other pituitary trophic hormones) is the normal condition encountered in the prepuberal child.

On the basis of the pathological physiology involved, correction of the resultant hypogonadism in all four of the above-mentioned circumstances depends upon stimulating the testis by the administration of FSH and ICSH. Application of this approach is best illustrated by 20 cases of hypogonadotropic eunuchoidism who were initially given chorionic gonadotropin (for its ICSH effect). This corrected the hormonal defect, but did not initiate spermatogenesis. The additional administration of a purified follicle-stimulating factor produced spermatogenesis. It was noted that, upon cessation of therapy, the hormonal

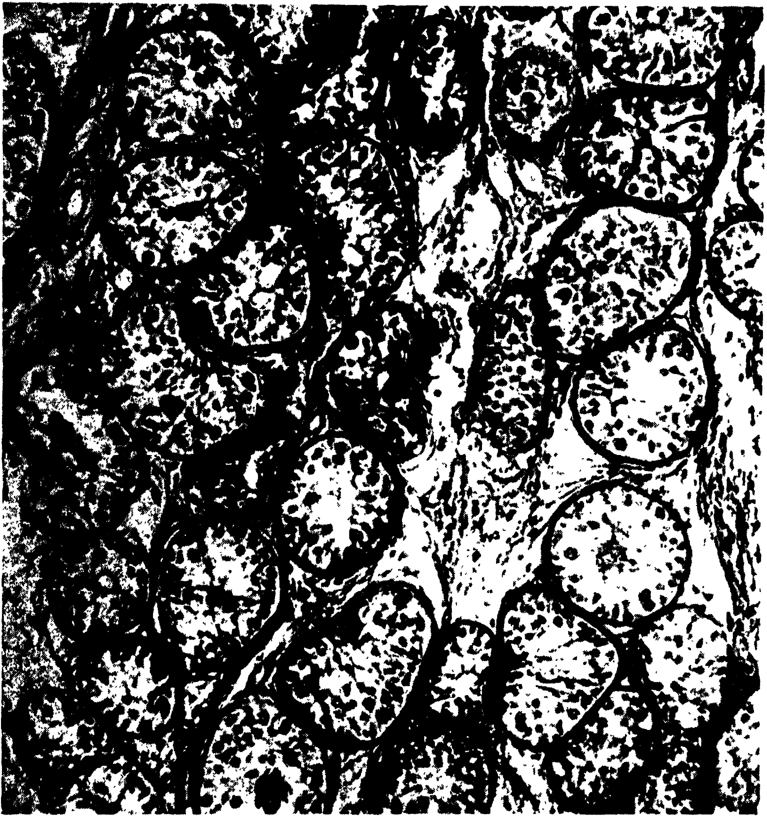


FIG. 2.—Section of testis from patient with hypogonadotropic eunuchoidism before treatment (× 175).

function of the testis, and occasionally the germinal function was maintained (Heller and Nelson, 1946). The effects upon the testes are illustrated in Figs. 2, 3 and 4 and should be contrasted with Figs. 5 and 6.

Since substitutional therapy by administering testosterone will correct the androgenic deficiency of each of the above four syndromes, the question arises: Is testosterone therapy advisable? Although not proved conclusively, it appears that testosterone from an exogenous source is harmful to the normal human testis (*vide infra*). It would seem quite probable that exogenous testosterone might more easily damage the unstimulated and deficient testis encountered in these syndromes (Figs. 5 and 6). It is our opinion that this unphysiological substitutional type

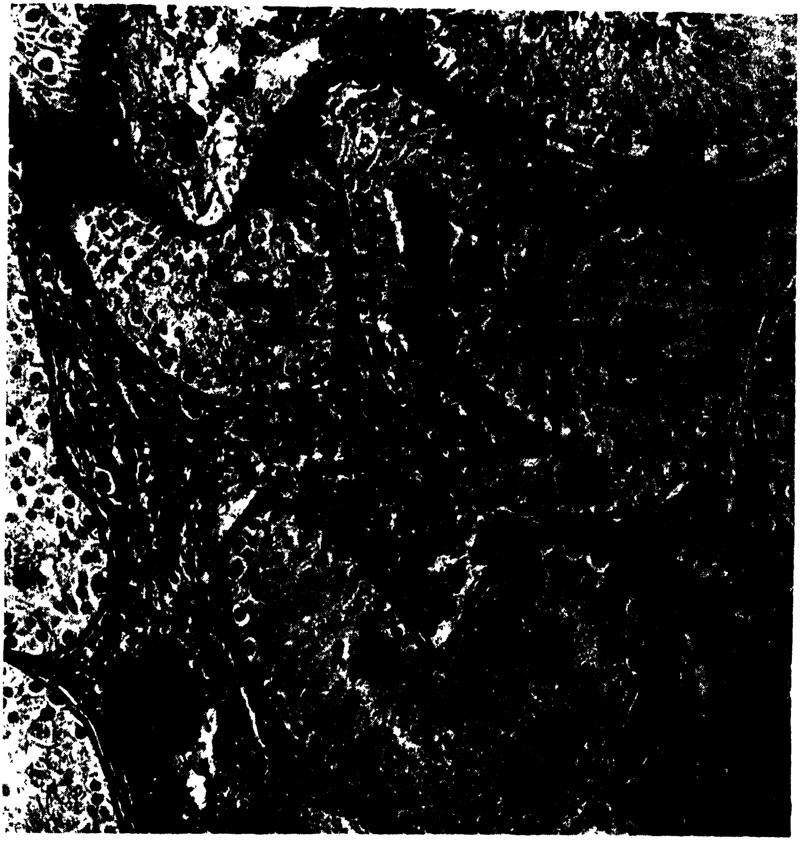


FIG. 3.—Section of testis from patient with hypogonadotropic eunuchoidism following treatment with chorionic gonadotropin. Note that the seminiferous tubules have increased greatly in size, but that spermatogenesis has not been stimulated (× 175).

of therapy is contraindicated, and that administration of gonadotropins is the treatment of choice.

b. In primary or hypergonadotropic hypogonadism the clinical features will depend upon such accompanying circumstances as (1) time of onset, *i.e.*, pre- or postpuberal and (2) portion of the testes primarily involved in failure, *i.e.*, Leydig cells or seminiferous tubules. The combinations of these two circumstances permit the existence of four distinct hypergonadotropic syndromes. These will include the following types of patients: eunuchoids, sterile individuals with gynecomastia, physically normal individuals with climacteric withdrawal symptoms and sterile individuals without signs of androgenic failure. These syndromes have in common high titers of gonadotropins and primary testicular pathology.

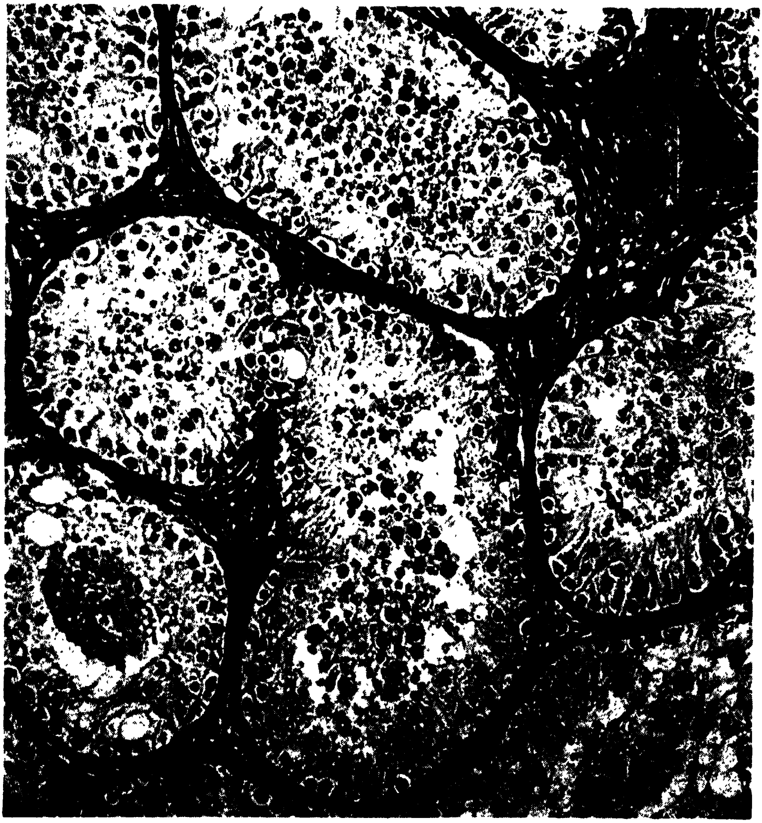


FIG. 4.—Section from same patient after treatment with both chorionic gonadotrophin and purified FSH. Note that spermatogenesis has been stimulated (× 175).

When the onset of failure occurs after sexual maturation has been attained, few clinically apparent evidences of hypogonadism are noted.

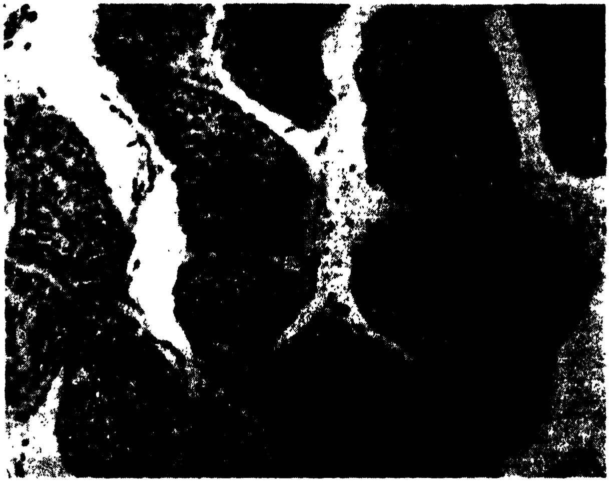


FIG. 5.—Section of testis from hypogonadal patient (× 150). (Charny and Meranze, 1942.)
(By permission of *Surg., Gynecol. and Obstet.*)

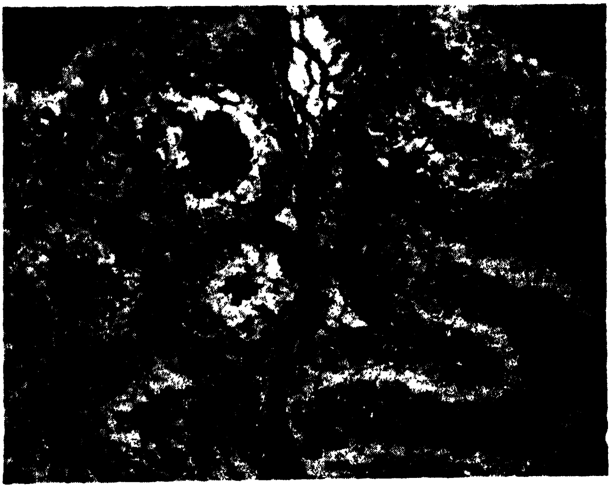


FIG. 6.—Section from same patient after treatment with testosterone propionate. Note the appearance of necrosis (× 150). (Charny and Meranze, 1942.)
(By permission of *Surg., Gynecol. and Obstet.*)

When the individual has Leydig cell failure or the entire testis is destroyed or rendered functionless by disease, developmental or mechanical processes, the most striking feature will be the androgen withdrawal symptoms of the male climacteric. When only the seminiferous tubules are affected, only the sperm content of the seminal fluid is abnormal.

When the onset of the failure occurs before or at puberty, and continues to be present as the age of puberty and adulthood are attained, skeletal changes or gynecomastia characterize the condition. In total testicular or Leydig cell failure eunuchoidal skeletal changes are accompanied by sexual infantilism. When the seminiferous tubules are involved primarily and the Leydig cells are affected minimally, the condition is characterized by gynecomastia, small testes and azoöspermia. The four hypergonadotropic syndromes are summarized in Table II. The hypergonadotropic syndromes are discussed in detail in the following section.

TABLE II

Hypergonadotropic Hypogonadism

Type of testicular deficiency	Time of onset	Condition
Entire testis functionless or Leydig cell failure	Before or during puberty	I. Hypergonadotropic eunuchoidism (Functional prepuberal castration) (Prepuberal castration)
	After puberty	II. Adult hypergonadotropic hypogonadism (Male climacteric) (Adult castrate)
Seminiferous tubule failure	Before or during puberty	III. Puberal seminiferous tubule failure (Klinefelter's syndrome)
	After maturation	IV. Adult seminiferous tubule failure (Sterility without signs of androgenic deficiency)

All of these syndromes (except group IV) respond favorably to testosterone administration.

4. *Details of the Hypergonadotropic Syndromes*

a. Hypergonadotropic Eunuchoidism. In its most complete form this results from surgical or functional castration prior to puberty.

Surgical Prepuberal Castration. Hamilton *et al.* (1945) demonstrated that orchiectomy performed prior to the onset of puberty results in a marked elevation of urinary gonadotropin titers and development of typical eunuchism in later years.

Functional prepuberal castration has been shown to be similar to surgical prepuberal castration in that the clinical appearance of the patients is the same, the urinary gonadotropin titers are elevated and the testes are absent or completely atrophic, although it is characteristic for the Wolffian duct derivatives to descend into the scrotum (Heller, Nelson, and Roth, 1943). It is most likely that both groups have changes in the microscopic appearance of the breasts. This has been noted for the functional castrates but to date no similar studies have been reported for the surgical castrates. Wagenseil's (1933) surgical castrates exhibited grossly observable gynecomastia similar to that observed in the functional group. Fraser, Forbes *et al.* (1941) describe three cases (their cases 67, 68 and 69) and Heller, Heller, and Sevringhaus (1941) note one case of eunuchoidism with elevated gonadotropin titers which most likely belong to this group.

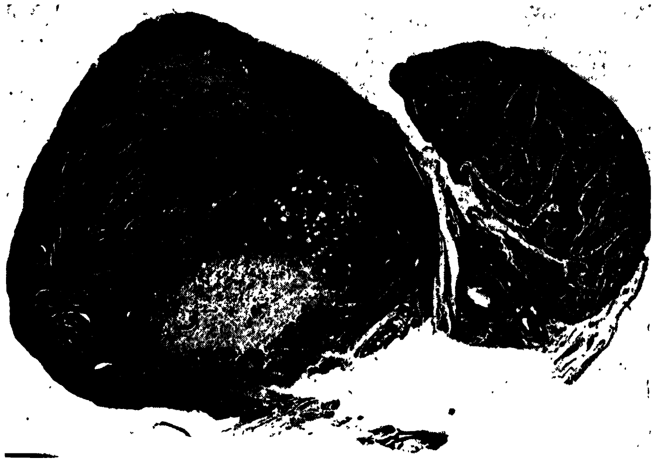


FIG. 7.—Section of scrotal contents of a case of functional prepuberal castration. Note thickened *tunica albiginea*, completely sclerosed seminiferous tubules, and presence of rete testis and epididymis (× 4). (Heller *et al.*, 1943.)

These patients have many clinical features resembling hypogonadotropic eunuchoidism. Separation is accomplished by the following procedures: (1) Gonadotropin titers make a clear-cut distinction. (2) Testicular biopsies reveal unstimulated but otherwise normal structures in hypogonadotropic eunuchoidism (Fig. 2) and only testicular remnants in functional prepuberal castrates (Fig. 7). (3) A therapeutic test may be substituted if the objective tests are unavailable. This consists of administering 750 international units of chorionic gonadotropin twice daily for 3 weeks to stimulate Leydig cells, if present. If a response is elicited, the presumptive diagnosis of hypogonadotropic eunuchoidism may be made; if no response occurs, it may be presumed that the testis is incapable of responding, the diagnosis of functional castration is established and substitution therapy with testosterone may be instituted.

(4) Mammary biopsies reveal no abnormality in the hypogonadotropic eunuchoid but in functional castration mammary abnormalities often occur. (5) Clinical observation may reveal these important differences;—(*a*) in the functional castrates—absence of testes in the scrotum or inguinal canals, often mistakenly considered cryptorchidism, and presence of gynecomastia, (*b*) in hypogonadotropic eunuchoidism,—soft, firm but small testes in scrotum, no gynecomastia.

Treatment by androgen substitution will produce the changes observed normally at puberty and convert the child-like state to the adult state as concerns secondary sex characteristics, muscular development and psychic adjustment. Androgen treatment does not affect the gynecomastia when present and will not produce fertility. Stimulatory therapy with gonadotropins is precluded by the absence of target-organ tissue capable of responding and by the presence of an excess of gonadotropins of endogenous origin.

b. Adult Hypergonadotropic Hypogonadism. This may result from surgical, mechanical or disease processes causing destruction of both functions of the testis or from disease affecting only the Leydig cells. In all instances sterility results, no skeletal structural change takes place, and regression of secondary sex characteristics occurs only after many years, but symptoms similar to those encountered in the female menopause may appear early.

Adult surgical castrates have been shown to have high titers of urinary gonadotropins years after orchiectomy (Hamburger, 1931; Fraser *et al.*, 1941 (cases 64 and 66); Frank and Salmon, 1936; Catchpole, Hamilton, and Hubert, 1942; Hamilton *et al.*, 1945). Scott and Vermeulen (1942) observed these changes in gonadotropic output in 3 cases and Heller and Myers (1944) in 6 cases before and after orchiectomy in elderly males castrated in an attempt to alter the course of their carcinoma of the prostate. Both groups detected normal preoperative levels of gonadotropins and noted sharp rises after castration. Heller and Myers (1944) observed an additional group of 6 cases with high postoperative titers. In none of the latter's 12 cases was overlapping with high normals noted.

"Male climacterics" or adult functional castrates are identical with the surgical castrates in that the titers of urinary gonadotropins are elevated to an equal degree. This is in distinct contrast to normal or neurotic aging men in whom urinary gonadotropins are not elevated (Fig. 8). As Saethre (1935), Heller, Heller, and Sevringhaus (1941) and Heller and Myers (1944) have shown, urinary gonadotropins remain at a stationary level throughout life (after puberty) in normal men. Nelson (1946) studied testes of aging men after orchiectomy for carcinoma of the

prostate and could not conclude that Leydig cell function was disturbed. This was in keeping with the normal gonadotropin and normal 17-ketosteroid titers of these same individuals. A study of 23 cases of the male climacteric (Heller and Myers, 1944) revealed that all had high gonadotropin titers, symptoms similar to the female menopause and impotence. In eight instances in which testicular biopsies were performed, abnormalities of the Leydig cells, although sometimes of a minimal degree, were observed. The menopausal-like symptoms were ascribed to androgen withdrawal because the testicular deficiency and the symptoms were associated and because testosterone (but not placebo) therapy reversed the symptoms. Additional evidence relating the symptoms to androgen withdrawal may be derived from the observation that such symptoms often follow surgical castration (Catchpole *et al.*, 1942; Scott and Vermeulen, 1942; Hamilton *et al.*, 1945). Testosterone administration abolishes the hot flashes in castrates (Catchpole *et al.*, 1942).

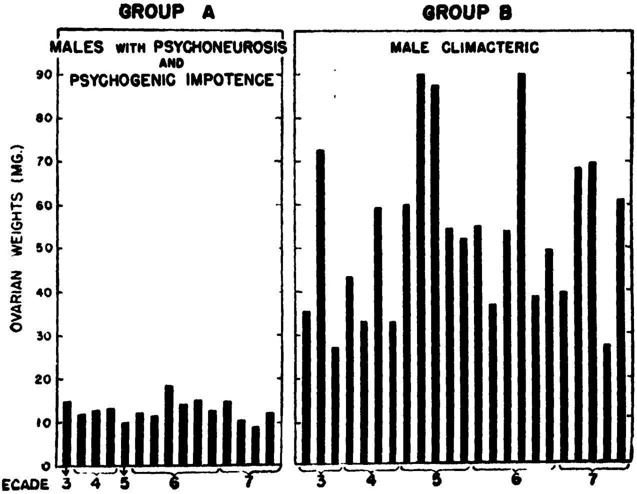


FIG. 8.—Gonadotropin assays of patients with male climacteric and with psychoneurosis and/or psychogenic impotence. Titers are expressed as ovarian weights of the assay animals. (Heller and Myers, 1944.)

The use of the term "male climacteric" has repeatedly been challenged. We justify its use on the basis of tradition, *i.e.*, men with menopausal-like symptoms have been thus referred to since Werner (1939) popularized the term. "Male climacteric" recalls the symptomatology of the female climacteric and suggests, quite correctly, the same origin, *i.e.*, cessation of active gonadal function. The term is odious if it connotes that the male climacteric is a normal accompaniment of the aging process in men. The conclusion drawn by Heller and Myers

(1944) concerning the occurrence of the male climacteric is: "whereas in the female the menopause is an invariable and physiologic accompaniment of the aging process, in the male the climacteric is an infrequent and pathologic accompaniment of the aging process."

c. Puberal Seminiferous Tubule Failure. Such failure was the first naturally-occurring hypogonadal syndrome in which the derangement in pathological physiology was described and a correlation made between the clinical findings and hormonal and biopsy findings (Klinefelter, Reifenstein, and Albright, 1942; Heller and Nelson, 1945a; Nelson and Heller, 1945; Heller and Nelson, 1945b; Reifenstein, 1946). It is the most common form of hypogonadism, but until described by Klinefelter *et al.* in 1942 had remained essentially unrecognized. This is partly due to the lack of pronounced and easily-recognizable clinical changes, and the infrequency with which these patients consult physicians with complaints directly referable to their hypogonadism.

It is difficult to ascribe a precise and informative name to the syndrome because of the variation in degree of Leydig cell involvement that is encountered in addition to the seminiferous tubule hyalinization. We personally refer to it as "Klinefelter's syndrome" but realize the disadvantage of such a designation. "Puberal seminiferous tubule failure" is correct but incomplete; correct, insofar as time of onset can be placed at or before puberty and that the seminiferous tubules are involved markedly in all cases and to more marked degree than the cells of Leydig; incomplete, insofar as the cells of Leydig are involved to minor or major degrees in all cases and treatment is directed toward correcting the resultant androgenic deficiency.

The syndrome can be characterized on the basis of its invariable features related to the seminiferous tubule failure, and subdivided on the basis of the variable features related to the degree of Leydig cell failure. The common denominators in each case include: (1) *Atrophic testes* (average size: 1½ × 1 × 1 cm.). Biopsy reveals hyalinization of the *tunica propria* of the tubules associated with failure of spermatogenesis and often the only intact cell is the supporting cell of Sertoli. Leydig cells are always present and tend to be aggregated into clumps. (2) *Azoöspermia* can be recognized in all cases in which ejaculation is possible. (3) *High gonadotropin* titers are always present.

A patient, admitted to the category because he has the above constant features, may be placed in one of the following three subdivisions: (1) eunuchoidal, (2) moderately eunuchoidal or (3) non-eunuchoidal (Fig. 9). The degree of Leydig cell function will determine the extent of androgen deficiency which, in turn, will be reflected in the number and extent of the variable features present. Note that the most marked gynecomastia is encountered in instances of best androgen production (Fig. 9).

It is now agreed that most cases derive distinct benefit from testosterone therapy (Heller and Nelson, 1945b; Reifenstein, 1946) in correction of the symptoms and stigmata of androgen deficiency, in whatever degree they may be present. However, neither the sterility nor the gynecomastia respond to hormonal therapy.

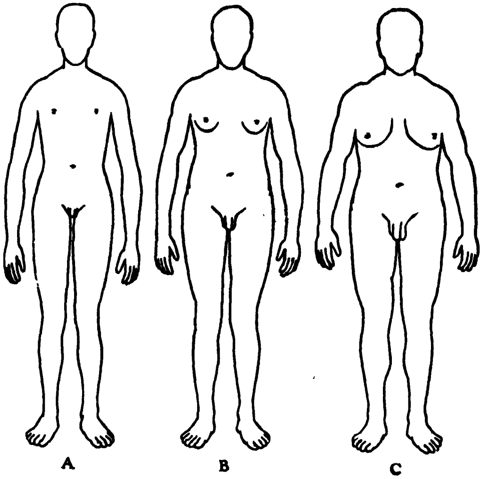


FIG. 9.—Schematic drawing representing the body configuration and genital and breast development of the three subdivisions of puberal seminiferous tubule failure (Klinefelter's syndrome). Note the absence of gynecomastia and the underdeveloped genitalia in the eunuchoidal group (A); the greater development of each in the moderately eunuchoidal group (B); and the association of marked gynecomastia and normal genital development in the non-eunuchoidal group (C). (Heller and Nelson, 1945a.)

d. Adult Seminiferous Tubule Failure. This may occur without Leydig cell involvement (Charny, 1944; Nelson and Heller, 1946, unpublished). The latter note the same degree of gonadotropin elevation as in the prepuberal group. In approximately one-third of the cases of adult seminiferous tubule involvement (hyalinization of *tunica propria* or simple failure of spermatogenesis), the Leydig cells are of poor quality as judged by their microscopic appearance. This is usually associated with androgen withdrawal symptoms. Testosterone therapy will correct these symptoms but will not promote spermatogenesis.

III. TESTOSTERONE ADMINISTRATION

Clinical investigation has narrowed therapeutically useful androgens to three chemical compounds administered by five routes. The compounds testosterone propionate, methyltestosterone and testosterone, have been administered by the following routes: intramuscular or subcutaneous injection, pellet implantation, inunction, oral and sublingual administration. The effectiveness of each compound varies with the route of administration. For example, testosterone propionate, while very active when given intramuscularly, is relatively inactive when given orally.

1. *Inunction*

Testosterone and testosterone propionate have been administered by inunction (Moore, Lamar, and Beck, 1938; Foss, 1938; Spence, 1940b; Emmens, 1941; Tager and Shelton, 1941). The amount of testosterone propionate used has varied from 5 to 40 mg. daily. According to Spence (1940b), testosterone is twice as effective as the propionate. The ointment is self-administered by rubbing a measured length of ointment into the skin of the thighs for 15 minutes, twice daily. Ointments are superior to alcoholic solutions as the vehicle (Foss, 1938; Zondek and Sulman, 1939; Spence, 1940b).

2. *Oral*

Methyltestosterone is the most potent orally administered androgen, presumably because the methyl group prevents enzymatic destruction of the active hydroxyl group in the alimentary tract (Miescher and Tschopp, 1938). It has also been suggested (Biskind and Mark, 1939) that the greater potency is due to selective absorption by lacteals, thus avoiding hepatic destruction. Clinical application has been made by administering 10–100 mg. daily in divided doses (Foss, 1939; McCullagh, 1940; Kearns, 1941a; Byron and Katzen, 1941; Escamilla and Lisser, 1941). These authors estimate that 2–6 times as much methyltestosterone as testoterone propionate I. M. is needed to achieve equal effects. Howard, Wilkins, and Fleischmann (1942) estimate that the androgenic effects are equal, whereas Heller and Myers (1944) find that 4–6 times as much methyltestosterone per weight was inadequate for satisfactory maintenance of male climacteric patients and that larger doses often caused nausea and vomiting and were too expensive to be practical. Others, too, have noted mild gastrointestinal symptoms, such as anorexia and nausea (Foss, 1939).

3. *Sublingual*

Methyl testosterone in the form of compressed tablets (Spence, 1942) and dissolved in propylene glycol (Lisser, Escamilla, and Curtis, 1942) has been administered sublingually in divided doses of 5–20 mg. daily. The tablets are reported by Lisser and Curtis (1943) to be more efficacious than the solution. The patient is instructed to hold the tablet under the tongue for the 15–30 minutes it takes the tablet to dissolve, and furthermore instructed not to bite, suck, swallow, talk or spit.

4. Intramuscular or Subcutaneous Injection

Such injection of testosterone propionate has been the classical means of administration. Kenyon (1938) states that 25 mg. daily seems to be a reasonable dose for complete replacement in the eunuch or eunuchoid. This has been the general experience of most workers. Often 25 mg. thrice weekly is sufficient as a maintenance dose.

5. Pellet Implantation

Compressed pellets of crystalline testosterone have been implanted subcutaneously with good effect (Deanesly and Parkes, 1937; Vest and Howard, 1939; Eidelsberg and Ornstein, 1941; Harding and Fraser, 1942). Methods of administration, size and number of pellets have varied. The most satisfactory method has been to insert cylindrical pellets by means of a trochar into the subcutaneous tissue of the medial aspect of the thigh. Adequate replacement is usually afforded by implanting three or four 75 mg. pellets into each thigh. The trochar method requires approximately 15 minutes for the insertion, entails no discomfort and needs be repeated every 6–8 months (Heller and Nelson, 1945b). The pellets are more effective per milligram than any other method of androgen administration because of the slower but more continuous delivery of the material.

Factors in the choice of treatment include expense, side-reactions, effectiveness, convenience to physician and patient, and assurance that the patient receives exactly the prescribed amount. Pellet implantation is by far the least expensive method currently available. Side-reactions are most common to oral and sublingual administration. All forms are effective if enough material is given. Sublingual administration and administration by inunction are convenient for the physician but very inconvenient to the patient. Injection is inconvenient for both patient and physician. Oral administration and implantation are convenient for both. Injection and pellet implantation are certain means that the dosage received equals the dosage prescribed. Pellet implantation must not be used in cases in which doubt as to the exact diagnosis exists or in patients in which a short period of action is desired, since, once implanted, no convenient way to stop delivery exists.

The two methods of choice are oral administration and pellet implantation. As yet, the former method remains too expensive.

IV. CHANGES EFFECTED BY ANDROGEN ADMINISTRATION

Androgen therapy produces changes in the secondary sex characteristics, voice, skin, genito-urinary system, and behavior of eunuchs and eunuchoids. These have been the subject of so many reviews, studies and case reports, that no useful purpose should be served to reiterate them here in detail. The order of response to adequate androgenic therapy usually progresses in a step-wise fashion, recapitulating the order of progression observed during normal maturation. The time relationship, however, is not the same. The general order of response may be summarized as follows: the number of erections are increased within the first week of therapy; ejaculation usually occurs by the third week, although the seminal fluid contains no spermatozoa. The voice begins to change in from 2–4 weeks. The genitalia begin enlarging by the tenth day and continue to increase in size for many months. The prostate gland increases to nearly normal size in 2–3 months. Evidence of increase in hair growth occurs in the following sequence: pubic, 2–4 weeks; lower arms and legs, 4–8 weeks; upper thighs and in line from pubic region to umbilicus, 3–4 months; axillary, general bodily and facial, 1–4 years. There is usually a decided change in personality, the patient changing from an antisocial to a more gregarious and aggressive individual.

Among the not so well-known changes attributable to androgen administration are nitrogen, creatine, and electrolyte retention, and elevation of basal metabolic rate. These are reviewed by Kochakian (1946) in Vol. IV of *Vitamins & Hormones.* Androgen administration tends to correct the nitrogen depletion which accompanies a wide variety of diseases and injuries in which no overt testicular deficiency is demonstrable. Feeding a high protein diet will also tend to correct the nitrogen depletion. Heller (1944) noted no consistant change in 17-ketosteroid, gonadotrophin or estrogen excretion which could be interpreted as reflecting gonadal deficiency in burned patients having a markedly negative balance. The nitrogen balance was converted to positive by feeding a high protein diet. Browne, Schenker, and Venning (1946) demonstrated that a high nitrogen intake alone is capable of converting a patient to positive balance. As testosterone administration and high protein feeding are both effective, the question arises: Which is the treatment of choice? Avoiding the use of testosterone seems to be desirable because it has potentially harmful effects upon the testis. Protein feeding seems to be a more physiological approach. If forced feeding fails, or if hypogonadism is present, the use of testosterone for its nitrogen-retaining effect seems justifiable.

V. EFFECT OF EXOGENOUS TESTOSTERONE ON SPERMATOGENESIS

Endogenous androgens play an integral part in spermatogenesis in that they maintain the seminiferous tubules, just as they maintain the other ductular structures of the male genital tract. Exogenous testosterone, however, does not always have this salutary effect. Testosterone will prevent the testicular atrophy following hypophysectomy in experimental animals (Nelson, 1940; Smith, 1944). However, exogenous testosterone causes testicular atrophy in intact animals of most species. Moderate amounts of testosterone will depress urinary gonadotrophin excretion (Heller and Nelson, 1945b). Atrophy of the testis could be explained by a reduction in pituitary gonadotropin secretion or to a direct depressant effect on the testis.

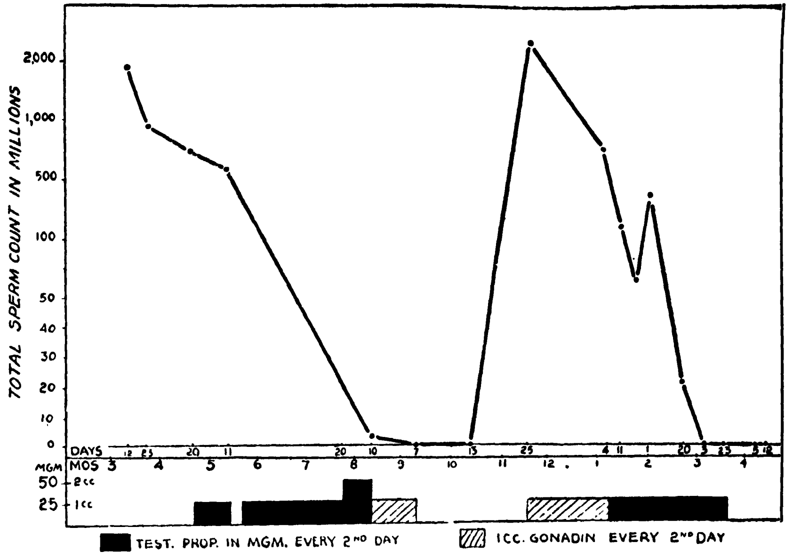


FIG. 10.—The effect of testosterone on the number of sperm. (McCullagh and McGurl, 1940.)

It has been clearly shown that exogenous testosterone markedly depresses the total number of sperm in normal men (Heckel, 1939, 1940, 1941; McCullagh and McGurl, 1939, 1940; McCullagh and Rossmiller, 1941) (Fig. 10). This effect is transient, since the number of sperm returns to previous levels when treatment is stopped. Whether the depression is permanent following prolonged administration of testosterone is not known. Rubinstein (1938) obtained a total sperm count of 8,400,000 on a hypogonadal patient after several months of treatment with testosterone propionate. He concluded that testosterone had stimulated spermatogenesis in this patient. It is difficult to conclude whether this single sperm count indicates a stimulation or depression of spermatogenesis as no pretreatment counts were performed. Rubinstein and Kurland (1939) report that 5 mg. of testosterone propionate given 3 times weekly increased the number of sperm in 5 normal men.

Testicular biopsies performed on patients after testosterone therapy suggest that the adverse effects of exogenous testosterone may persist after cessation of treatment. Heckel (1941) found that, following administering testosterone propionate, the total number of sperm, which averaged 287,000,000 before treatment, dropped to 17,000,000. Testicular biopsies performed at this time revealed atrophy and degeneration of the seminiferous tubules (Fig. 11). Charny and Meranze (1942) performed testicular biopsies on a hypogonadal patient before and after therapy with testosterone propionate. They noted necrotic changes in the seminiferous tubules, similar to that induced by stilbesterol (Figs. 5 and 6).

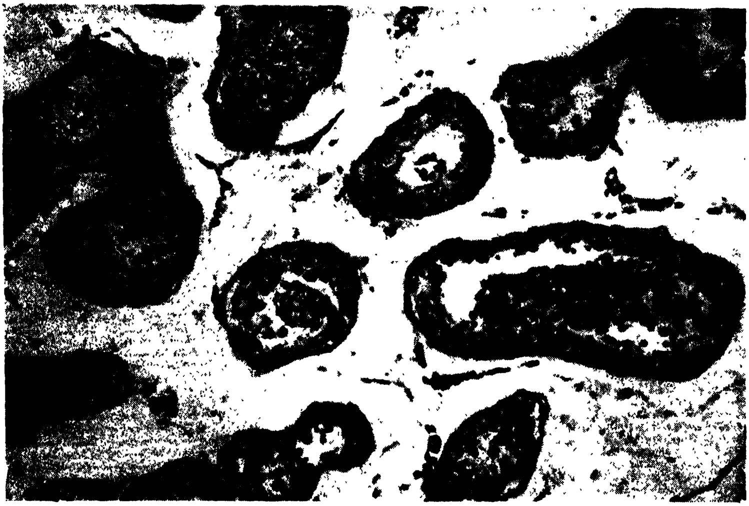


FIG. 11.—Section of testis from a patient who had received 2,430 mg. testosterone propionate. Before treatment, the sperm count was 287 million. After treatment, at the time of the testicular biopsy, the sperm count was 17 million. Note atrophy and degeneration of the seminiferous tubules. (Heckel, 1941.)

In the light of currently available information it is highly probable that nothing but harm will result from treating sterility with testosterone. Until it has been established that prolonged administration of testosterone does not result in permanent damage, it would seem wise to limit the use of testosterone to those individuals in which the preservation of fertility is not a factor to be considered.

VI. USE OF TESTOSTERONE IN THE PREPUBERAL CHILD

Androgen therapy is specifically contraindicated in the preadolescent child. As has been pointed out in several sections of this review, the prepuberal boy is by definition hypogonadal, *i.e.*, the testis does not secrete androgen and it does not produce sperm. Therefore, variations in height, in size of penis, in fat distribution, in bone age, and such variations in skeletal configuration as *genu valgum* cannot logically be attributed to androgenic deficiency. Such variations have often been classified as adiposogenital dystrophy or Fröhlich's syndrome[3] under the general heading of hypogenitalism, and treatment with androgens has been instituted on the basis of these anthropometrical observations.

Treatment with testosterone before the age of puberty is deemed inadvisable because of the premature maturation it occasions, the possibility of early closure of epiphyses which may lead to dwarfism, and the possibility of causing testicular damage which may or may not lead to permanent sterility (McCullagh and McGurl, 1940).

If, at the average age of puberty, no signs of sexual development are observed, the problem arises: Is this patient an instance of delayed adolescence or is he truly hypogonadal? Waiting for a number of years may elicit an answer. However, waiting has the disadvantage that the youth will be considered "different" by his colleagues and will react to this psychogenically. To obviate such difficulties, some therapy directed toward speeding sexual maturation may seem desirable. The therapy with least disadvantage would be stimulatory treatment with gonadotropins.

VII. CRYPTORCHIDISM

For an exhaustive review and an extended bibliography the reader is referred to the article by Bishop (1945). Innumerable suggestions as to kinds of hormonal therapy, time for surgery, and harmful effects of "watchful expectancy" along with many acrimonious arguments have been forthcoming. After considering the evidence presented for each particular approach, the following summary of the situation can be made: (1) The majority of preadolescent boys with cryptorchidism and/or pseudocryptorchidism will attain spontaneous descent of the testis at the time of puberty (Drake, 1934; McCutcheon, 1938; R. E. Smith, 1939; W. W. Johnson, 1939; Mimpriss, 1945; Fruin, 1945). (2) Administering androgens, chorionic gonadotropin and pregnant mare serum, singly or in combination, before the onset of puberty will cause testicular descent in approximately the same percentage of patients as have spontaneous descent. (3) The undescended testis before puberty is indistinguishable from the normal prepuberal testis in both its macroscopic and microscopic appearance (Rea, 1939; Wangensteen, 1932; Eccles, 1902; Felizet and Branca, 1902; Rawlings, 1908; Southam and Cooper, 1927; Pace, 1935), indicating no harm results from leaving it in an ectopic position until puberty. (4) The retained testis generally shows marked involution of its germinal elements after puberty is attained to the extent that in adulthood, spermatogenesis rarely occurs. Rea (1939, 1942) summarizes the literature pertinent to this subject, and notes that, with increasing age after puberty, progressive seminiferous tubule destruction occurs.

---

[3] This term is correctly used in referring to a rare syndrome in which the symptoms of hypothalamic involvement are the outstanding characteristics.

Androgens, or androgens and chorionic gonadotropin combined, have been administered, with or without success, to preadolescent boys with cryptorchid testes by Denk and Uebelhoer (1936), Zeithaml (1938), Webster (1938), Hamilton and Hubert (1938), Zelson and Steinitz (1939, 1940), Jaffe and Brockway (1942), Rubinstein (1942), and Harding (1943).

We suggest the following for the treatment of uncomplicated cryptorchidism: Use no endocrine therapy and perform no surgery during the prepuberal period. As the onset of puberty varies greatly in normal children, no definite chronological age for intervention can be set. However, a physiological end-point for the onset of puberty may be used; namely, the development of penis and scrotum, and the appearance of pubic hair. If the above signs of puberty appear, without descent taking place, it may be assumed that the testis is being mechanically retained. At this point, surgical procedures for fixing the testis in the scrotum are indicated. Any further delay may result in testicular damage. The use of endocrine preparations as a preoperative measure for facilitating surgery seems unnecessary in view of the fact that the testis is already elaborating sufficient hormone to induce puberty and thereby to induce the necessary development of the scrotum and elongation of the cord, which is a prerequisite for a successful operation. It must be emphasized that surgery should neither be performed before nor appreciably after the onset of puberty, but *during* the early stages of puberty.

VIII. IMPOTENCE

Sexual drive is a resultant of two forces, hormonal and psychogenic. Conversely, impotence may result from hormonal or psychogenic disorders. It is generally agreed that the impotence associated with hypogonadism may be corrected by androgen administration. It is also agreed that impotence may be due to psychogenic factors without an associated hypogonadism.

Optimistic reports concerning the use of testosterone in patients with psychogenic impotence and impotence of unknown origin have been made by Huhner (1939), Hamilton (1937), Miller, Hubert, and Hamilton (1938), Reed and McMillan (1941) and Thomas and Hill (1940).

On the other hand, the careful studies of Rennie, Vest, and Howard (1939), Spence (1940a), Creevy and Rea (1940b), and Carmichael, Noonan, and Kenyon (1941) cast serious doubt upon the assumption that testosterone therapy is specific for psychogenic impotence. The latter found that those cases that seemed to respond to testosterone injections responded just as well to placebo injections. Kenyon (1941) suggests that only individuals with overt testicular insufficiency will respond.

Gonadotropin assays have been found readily to delineate psychogenic impotence (normal titers) from impotence due to overt testicular failure in the male climacteric (high titers) (Heller and Myers, 1944). The gonadotropin titers of these two groups of impotent males are compared in Fig. 8. Men in the psychogenic group failed to respond to testosterone therapy whereas the impotence was corrected by testosterone in each of the climacteric cases.

It is not unanticipated to find that hormonally normal impotent men do not respond to testosterone since normal men with normal sexual potency fail to have an increase in sex drive when excesses of exogenous androgen are administered (Rubinstein and Kurland, 1939; Rennie, Vest, and Howard, 1939; Kenyon, Knowlton *et al.*, 1940). It may be concluded that testosterone therapy is specifically indicated in instances of impotence due to Leydig cell failure and specifically contraindicated in instances of psychogenic impotence, for, besides not correcting the impotence, testosterone may induce sterility, at least temporarily, by depressing sperm production. The importance of clearly separating these two types of impotence must, therefore, not be underestimated.

IX. Homosexuality

A widely-held opinion is that homosexuality is caused by an endocrine imbalance. The vague generality that glandular dysfunction causes homosexuality was the contention of such early students as Krafft-Ebing (1892), Hirschfeld (1940), Bloch (1928) and H. H. Ellis (1936), as well as many others mentioned by A. Ellis (1945). The position of those believing in endocrine origin of homosexuality has gradually crystallized to the point that recently the majority ascribe it to an "estrogen-androgen imbalance" (Wright, 1935, 1938, 1939, 1941; Glass, Deuel, and Wright, 1940; Glass and McKennon, 1937; Glass and Johnson, 1944; Myerson and Neustadt, 1942a,b; Neustadt and Myerson, 1940; Sevringhaus and Chornyak, 1945).

A totally opposite opinion was held by such early students as Freud (1924), Stekel (1933), Brill (1913) and Adler (1917) who viewed homosexuality as arising primarily from psychogenic maladjustments. More recent investigators who hold to the same view include Henry (1934), Beach (1944), and Kinsey (1941).

Among those who have subscribed to the opinion that homosexuality is due to an estrogen-androgen imbalance, some have naturally taken the step of applying androgenic therapy to their cases. Androgen administration has been claimed successful in some cases (Wright, 1941; Myerson and Neustadt, 1942b; Glass and Johnson, 1944; Lurie, 1944). Failure of androgenic therapy has been reported by Rosenzweig and Hoskins (1941). Diametrically opposed to this type of therapy is the use of castration in over 100 cases of sexual perversion and homosexuality reported by Sand and Okkels (1938) who note that the results have been gratifying in all but one case.

Such a dichotomy of opinion concerning etiology and treatment is quite characteristic of the current status of numerous endocrine problems. Therefore, an analysis of the problem of homosexuality might prove illuminating.

The problem can be divided into two general phases: (1) Incidence of homosexuality in conditions known to be associated with excessive or decreased amounts of sex hormones, and (2) evidence for and against the belief that homosexual individuals have any deviation from normal in sex hormone physiology.

From the above claims it might be expected that conditions characterized by a reduction in androgen in the male such as the male climacteric, eunuchism and eunuchoidism are often associated with homosexuality. This is definitely not so, although occasionally passive homosexuality may be encountered. The frequency of the latter is certainly no greater than in the general population. In the course of questioning and examining over 300 cases of male hypogonadism one of us (CGH) encountered but a single instance, and that of passive homosexuality in a mentally subnormal eunuchoid.

Artificially altering the "androgen-estrogen ratio" in males by the administration of large doses of estrogen is now common practice in the management of carcinoma of the prostate. If the claims for an endocrine imbalance in homosexuality are correct, then it might be expected that these individuals would develop an abnormal direction of their sex drive. This is decidedly not the case. No instance of such an occurrence has been reported. Likewise, females having virilizing tumors of the ovary or adrenal glands, although they develop an altered estrogen-androgen ratio, do not develop male sex interest. Similarly, the administration of androgens to females causes no aberration in sexual interest.

In his recent review of the sexual psychology of human hermaphrodites A. Ellis (1945) stresses the importance of the environment in determining the direction of the sex drive. He concludes that "in the great majority of cases, the hermaphrodite assumes a heterosexual libido and sex role that accords primarily not with his or her internal and external somatic characteristics, but rather with his or her masculine or feminine upbringing." Thus it can be concluded that the incidence of homosexuality in conditions known to be associated with excessive or decreased amounts of sex hormones is no greater than in the population as a whole.

The second phase concerns itself with reviewing the evidence for the belief that homosexuals have any significant deviation from normal in sex hormone physiology. The likelihood of this possibility is indeed small when one considers that homosexuals usually undergo a normal puberty at a normal time. This can be inferred from the developmental history of the individual as well as by anthropometrical data obtained after maturation. Homosexuality cannot be discovered by physical examination, as the muscle mass, hair distribution, voice, external genitalia, and other secondary sex characteristics are indistinguishable from normal. Histological examination of the testes of such cases (Sand and Okkels, 1938) reveals no deviation from normal. Sexual function seems normal enough when one considers that these individuals are capable of having normal erections, seminal emissions, and when they indulge in heterosexual relationships are perfectly able to conceive. The only feature which distinguishes these patients from normal is the *direction* of their sexual behavior.

Despite all this evidence to the contrary, the postulation that homosexuality has its origin in hormonal imbalance has remained so persistent that numerous investigators have resorted to hormonal analysis to settle the question. Even though these objective measurements have been made, the opinion of those making them remains divided.

Brahn (1931) and Österreicher (1934) find that the urinary estrogen levels in homosexual patients are within normal limits. Sevringhaus and Chornyak (1945) find that urinary 17-ketosteroids in homosexual patients are also normal.

Wright (1941), Glass, Deuel, and Wright (1940), and Neustadt and Myerson (1940), have resorted to indicting the estrogen-androgen ratio as being an objective measure of difference between normal and homosexual individuals. They imply that this change in ratio in homosexuals accounts for the altered behavior. It is difficult to analyze the various reports of Myerson and Neustadt because they do not reveal the results of the individual assays of normal subjects. Wright's work is difficult to interpret since, in his reports, he fails to use normal controls. As a consequence, the paper that lends itself best to critical analysis is that of Glass, Deuel, and Wright (1940). This has been done by Kinsey (1941), who, after correcting the mathematical errors found in their paper, has statistically analyzed their data and concludes: "One is not warranted in concluding that the relatively small differences in averages between two such small groups are significant when successive samples from single individuals show from 7 to 50 times as much difference." He further criticizes their method of sampling. Kinsey finds that by one method of sampling their data, the homosexual seems to show more androgen than the normal group; by another method of sampling there is less androgen. Kinsey concludes that whatever the method of sampling, the differences between normals and homosexuals involved such large standard errors that little significance can be attached to the results.

It may be inferred from the above discussion that the *power* of the human sex drive is largely dependent on *physiological* factors, *i.e.*, proportional to the amount of circulating androgen (or estrogen in the female). On the other hand the *direction* of the human sex drive seems to be largely dependent upon *psychological* factors, which are conditioned by the early environment and sexual experiences of the individual. It would seem to follow that vigorous androgenic treatment would tend to increase the power of the sex drive in both normal and homosexual males without influencing the direction of the sex drive in either case. Similarly, castration will markedly diminish, but not abolish, the power of the sex drive, and the diminished drive, in the case of normals will continue in the direction of heterosexuality, whereas the small drive remaining in the homosexual will continue in the direction of homosexuality.

X. Angina Pectoris

Since 1939, when Arndt reported that intramuscular administration of testosterone propionate was effective in alleviating the symptoms of angina pectoris, many reports of the use of androgens for this disease have been forthcoming.

Among the most enthusiastic and optimistic supporters of this form of treatment for angina pectoris are the following: Walker (1940, 1942) who reports definite improvement in 11 of 14 cases of angina pectoris using testosterone propionate; Bonnell, Pritchett, and Rardin (1941) who reported "clinical improvements in 22 out of 23 patients" with coronary artery disease following administration of estrogens and/or androgens; Lesser (1942, 1943) reports 100% of 46 patients with angina pectoris responded favorably to administration of testosterone propionate, 25 mg. intramuscularly every 2–5 days. He claimed that the beneficial effects of treatment persisted 10–12 months after treatment was discontinued. Sesame oil placebos were ineffective. He later reports (Lesser, 1946) a series of 100 cases of angina pectoris treated with testosterone propionate. Ninety-one per cent improved for periods ranging from 2 to 34 months. Hamm (1942) also reports uniformly beneficial results from androgen therapy in 7 out of 7 cases. These results are astonishing when one considers how few chronic diseases respond favorably 100% of the time to a given therapeutic agent. They are even more astonishing when one considers the poor response generally expected in angina pectoris. Riseman (1943) conducted a 10-year study of treatment in 75 patients with angina pectoris, testing 68 commonly used agents. He concludes that 40% of patients usually fail to respond appreciably to *any* form of therapy.

Less enthusiastic, but reporting favorable responses to androgen treatment, are Sigler and Tulgan (1943) who observed "unmistakable evidence of symptomatic relief from the anginal syndrome" in 11 cases, and "less spectacular" relief in 5 additional cases of their 20 cases treated with testosterone propionate. They note no improvement in the electrocardiographic findings and admit "other factors may be responsible" for the relief obtained. Strong and Wallace (1944) demonstrated some improvement in 17 of 20 cases and Waldman (1945) reports 7 of 10 benefited.

The first doubt cast upon this form of therapy was by Riseman (1943) who investigated and classified various therapeutic agents claimed to be of aid in alleviating anginal pain, as to their "degree of value." He included testosterone (25 mg. daily) among 27 agents he placed in the "none" category in his table of the comparative value of methods of treatment. Shortly thereafter S. A. Levine and Likoff (1943) were unable to conclude from observations made on 19 cases "that testosterone propionate has any beneficial therapeutic effect in this disease." They ascribe the improvement noted in 5 of their treated patients to the spontaneous variation of the clinical course of angina pectoris. Hurxthal and Wilson (1944) reported improvement in 6 out of 19 cases of angina pectoris. Testosterone was administered by intramuscular injection, sublingual tablets, and subcutaneous pellet implantation. They summarize their observations as follows: "The disappointing results of pellet implantation in 3 cases, in which, apparently, benefit should have been forthcoming if it was to be obtained from any form of testosterone therapy, led us to believe that there was little of value in this treatment. While one patient thought he was benefited, he also received benefit from placebo treatment. Since these pellets are easily and quickly implanted by trochar and require no further attention, psychic effect might be considered less when compared with the daily self administration of drops sublingually or intramuscular injections 2 or 3 times a week." E. Levine and Sellers (1946) concur with this view. Their experience leads them to conclude that testosterone is of no value in the treatment of true angina pectoris.

Discussion of these contradictory results is especially merited in view of the widespread use of androgens for the treatment of angina pectoris.

In evaluating the efficacy of a therapeutic agent, one should inquire as to the correctness of the diagnosis and the objectivity of the end-points by which the response to therapy is judged. By no means have all of the authors of the above-mentioned articles definitely established that their patients were suffering from angina pectoris. Precordial pain of non-cardiac origin is associated with other syndromes such as the anxiety tension states and the male climacteric. The "angina-like" pain of the climacteric responds to substitutional therapy with testosterone (McGavack, 1943; Heller and Myers, 1944; E. Levine and Sellers, 1946). It would not be surprising to find that the precordial pain associated with some of the psychoneuroses would respond to such psychotherapy as is afforded by frequent injections. Similarly the usual clinical course of angina pectoris, characterized as it is by spontaneous exacerbations and remissions, must be taken into account in evaluating treatment.

Objective tests for judging the severity of angina pectoris are difficult to devise because of the inherently subjective nature of the syndrome. Even the standard exercise tolerance test depends for its end-point upon the purely subjective experience of pain on the part of the patient. The electrocardiogram, although objective, is not a measure of the severity of angina pectoris. Subjective improvement alone, therefore, must be depended upon as the criteria for judging the efficacy of testosterone in treating angina. In addition, it would be helpful to know whether such relief as is afforded could be ascribed to any of the known actions of testosterone.

Lack of this hormone due to primary testicular failure is an undisputed indication for its use. It would add substantially to the credibility of the reports if it were demonstrated that androgen deficiency existed in angina pectoris. However, few have claimed, and none have proved, that such a deficiency exists. Conversely it is not characteristic for eunuchs and eunuchoids to have angina pectoris. Therefore, any effect of testosterone in angina pectoris cannot be explained on the basis of correcting an hormonal deficiency.

The theory most often advanced by the proponents of its use in angina pectoris is that it has a direct action on the coronary vessels. To date, neither clinical nor experimental evidence supports this concept. The incidence of intimal thickening of coronary arteries and the incidence of coronary disease is so much greater in males than in females (Dock, 1946) that it suggests testosterone might actually predispose to coronary disease.

To clarify the problem it would be of interest to know the effect of testosterone on coronary blood flow. We suggest that both acute and chronic effects be investigated in experimental animals, in normal as well as in damaged hearts.

XI. Benign Prostatic Hypertrophy

Over 100 publications concerning the efficacy of testosterone as a therapeutic agent in benign prostatic hypertrophy have appeared to date. In contradistinction to its use in many diseases, there was a large body of experimental work concerning the relation of the endocrine system to the prostate preceding the clinical use of testosterone in prostatic hypertrophy. From this work have evolved theories implicating the endocrine system in the etiology of this disease.

It has been shown that administration of estrogens to mice results in metaplasia of the prostatic epithelium (Lacassagne, 1933; Burrows, 1934). As metaplasia of the ductular epithelium is a common finding in hypertrophy of the human prostate, it was tentatively concluded that increased estrogen activity was a possible cause of this condition (van Cappelen, 1936; Zuckerman, 1936). Zuckerman and Parkes (1936) demonstrated that injecting androgens along with estrogens would prevent the metaplastic changes in the prostate of a monkey induced by estrogen administration. From this evidence alone, it would appear that there are definite possibilities for testosterone therapy in prostatic hypertrophy.

There are two important facts that detract from the application of these observations to the problem of human prostatic hypertrophy. The first has been aptly stated by Moore and McLellan (1938): " . . . the histological changes produced by estrogens in mice, except for metaplasia, do not have even the slightest resemblance to the disease in man. The characteristic and pathognomonic formation of nodules with encircling glands is never seen in the experimental animals." The second is that urinary estrogen determinations have not disclosed an increase in estrogen excretion (Hamilton, Deming, and Allen, 1936; Owen and Cutler, 1936; Dingemanse and Laqueur, 1940). It should be emphasized that the effect of androgens in the normal human male is to bring about a marked increase in size of the prostate at puberty, and after puberty, to maintain the prostate in the mature state.

Nonetheless, many optimistic reports have been forthcoming in the literature (Strohm, Edelson, and Merryman, 1938; Trasoff, 1940; Markham, 1941; Soifer, 1941, and many others). The majority have noticed no changes in objective signs, such as diminution in prostate size, but have reported subjective improvement on the part of the patient. Moore and McLellan (1938) and Heckel (1940) found no gross or histological changes in the prostate of cases with benign hypertrophy after administering testosterone propionate.

Lippross (1938), Kearns (1941b), Egger (1944), and Muellner and Hamilton (1944) reported that the injection of androgens resulted in an increase in bladder tonus in men suffering from benign prostatic hypertrophy, suggesting that the subjective improvements in patients so treated may be explained on this basis. Whether or not this effect is a true pharmacological action of testosterone on the bladder musculature, or merely the effect of replacing an hormonal deficiency has not been clearly defined as yet. That an increase in bladder tonus would be effected in all patients is not likely in view of the report of Heckel (1940), who noticed no "noteworthy reduction" in the residual urine of 22 patients treated with testosterone propionate.

An even more plausible explanation for the subjective improvement in patients treated with testosterone is the psychotherapeutic effect of frequent injections of this substance. Draper, Slaughter, and Denslow (1941) in a terse summary of their results, state: "We have observed no apparent improvement in the urinary status of our cases of early benign prostatic hypertrophy resulting from injection of testosterone which could not be duplicated by injection of sterile saline." Creevy and Rea (1940a) conclude: "It remains to be demonstrated that the injection of testosterone propionate into patients with benign hypertrophy of the prostate is more than a form of psychotherapy." Heckel (1940) reports: "The benefits that did occur were mild, and were no greater than the improvement commonly seen when the patient is prescribed soda and sitz baths."

It may be that testosterone, if applied routinely as a therapeutic measure in prostatic hypertrophy, could be harmful. It is well known that the direct antithesis of testosterone therapy, *i.e.*, castration, is of definite value in carcinoma of the prostate. Furthermore, it has been demonstrated that testosterone exerts an unfavorable influence in prostatic cancer (Huggins and Hodges, 1941). Assuming that a definite number of patients diagnosed as having benign prostatic hypertrophy, will actually have, or have in addition, carcinoma of the prostate, the deleterious effects of routine testosterone therapy are obvious.

Realizing that prostatic hypertrophy is a disease characterized by exacerbations and remissions, Riches (1938) advances this as an explanation for a certain percentage of good results obtained by any method. He warns against the use of testosterone, reasoning that apparent good results may delay a needed operation. Later, when the patient is older, the operation becomes mandatory at a time when the patient is less well suited for it.

Believing that the good results obtained with testosterone in the therapy of prostatic hypertrophy are more apparent than real, and feeling that its use may cause harm, the reviewers conclude that there is no indication for its use in benign hypertrophy of the prostate.

XII. CONCLUSIONS

Androgen therapy causes a specific and desirable response (sometimes a dramatic metamorphosis) in certain situations; in other situations it elicits a response in which it is not the drug of choice, and in a third situation, the response elicited is highly undesirable. These situations include: (1) the specific, marked and desirable response that follows its administration in the following hypergonadotropic hypogonadal syndromes; hypergonadotropic eunuchoidism (functional and surgical prepuberal castration), adult hypergonadotropic hypogonadism (male climacteric and adult castrate) and the puberal seminiferous tubule failure syndrome (Klinefelter's syndrome). (2) The situations in which testosterone may produce a response but is not the drug of choice are cryptorchidism, delayed adolescence and the hypogonadotropic hypogonadal syndromes: panhypopituitary dwarfism, Simmond's cachexia, hypogonadotropic eunuchoidism and adult hypogonadotropic hypogonadism. (3) The third situation in which a response may be elicited, but such a response is highly undesirable, is following its administration to preadolescent children.

There are a number of situations in which testosterone has been repeatedly applied and found to have no specific beneficial effect and in which it may even prove to be harmful. These include the use of testosterone in psychogenic impotence, homosexuality, angina pectoris and benign prostate hypertrophy. Probably its use in male infertility should be included in this category, but, at present, information is too insufficient to be conclusive. Testosterone has been used in many other conditions such as tinea capitis infestation, peptic ulcer, hypertension and diabetes mellitus in which little benefit from its use could be expected.

Use of steroid hormones for their protein anabolic effects is being systematically investigated in many qualified laboratories and answers should be forthcoming in the near future.

XIII. SUMMARY

Testosterone has a single undisputed use. This is its application in correcting the signs and symptoms that attend gonadal failure due to primary testicular involvement.

We wish to express our appreciation to Mrs. Margaret Wolff for her help with the manuscript.

REFERENCES

1. Adler, A. 1917. *Alienist & Neurol.* **38,** 268–287.
2. Arndt, H. 1939. *Wien. med. Wochschr.* **89,** 222–227.
3. Aub, J. C., and Kety, S. S. 1943. *New Engl. J. Med.* **228,** 338–343.
4. Beach, F. A. 1944. *J Clin. Endocrinol.* **4,** 126–134.
5. Bishop, P. M. F. 1945. *Guy's Hosp. Repts.* **94,** 12–74.
6. Biskind, G. R., and Mark, J. 1939. *Bull. Johns Hopkins Hosp.* **65,** 212–217.
7. Bloch, I. 1928. The Sexual Life of Our Time. Allied Book Co., New York.
8. Bonnell, R. W., Pritchett, C. P., and Rardin, T. E. 1941. *Ohio State Med. J.* **37,** 554–556.
9. Brahn, B. 1931. *Klin. Wochschr.* **10,** 504–505.
10. Brill, A. A. 1913. *J. Am. Med. Assoc.* **61,** 335–340.
11. Browne, J. S. L., Schenker, V., and Venning, E. H. 1946. *Fifty-ninth Ann. Meeting Assoc. Am. Physicians.*
12. Burrows, H. 1934. *Nature* **134,** 570.
13. Byron, C. S., and Katzen, P. 1941. *J. Clin. Endocrinol.* **1,** 359–362.
14. Callow, N. H., and Callow, R. K. 1940. *Biochem. J.* **34,** 276–279.
15. Callow, N. H., Callow, R. K., and Emmens, C. W. 1940. *J. Endocrinol.* **22,** 88–98.
16. Carmichael, H. T., Noonan, W. J., and Kenyon, A. T. 1941. *Am. J. Psychiat.* **97,** 919–943.
17. Catchpole, H. R., Hamilton, J. B., and Hubert, G. R. 1942. *J. Clin. Endocrinol.* **2,** 181–186.
18. Charny, C. W. 1940. *J. Am. Med. Assoc.* **115,** 1429–1432.
19. Charny, C. W. 1944. *J. Med. Sci.* **207,** 519–527.
20. Charny, C. W., and Meranze, D. R. 1942. *Surg. Gynecol. Obstet.* **74,** 836–842.
21. Council on Pharmacy and Chemistry. 1939. *J. Am. Med. Assoc.* **112,** 1949–1951.
22. Creevy, C. D., and Rea, C. E. 1940a. *Urol. and Cutaneous Rev.* **44,** 430–439.
23. Creevy, C. D., and Rea, C. E. 1940b. *Endocrinology* **27,** 392–394.
24. Deansely, R., and Parkes, A. S. 1937. *Proc. Roy. Soc. London* **124,** 279–298.
25. Denk, W., and Uebelhoer, T. 1936. *J. intern. chir.* **1,** 369.
26. Dingemanse, E., Borchardt, H., and Laqueur, E. 1937. *Biochem. J.* **31,** 500–507.
27. Dingemanse, E., and Laqueur, E. 1940. *J. Urol.* **44,** 530–540.
28. Dock, W. 1946. *J. Am. Med. Assoc.* **131,** 875–878.
29. Drake, C. B. 1934. *J. Am. Med. Assoc.* **102,** 759–761.
30. Draper, J. W., Slaughter, G., and Denslow, C. 1941. *J. Urol.* **45,** 539–547.
31. Eccles, W. M. 1902. *Brit. Med. J.* **1,** 503–505.
32. Egger, K. 1944. *Schweiz. med. Wochschr.* **74,** 676–680.
33. Eidelsberg, J., and Ornstein, E. A. 1941. *J. Am. Med. Assoc.* **117,** 1068–1074.
34. Ellis, A. 1945. *Psychosomat. Med.* **7,** 108–125.
35. Ellis, H. H. 1936. Studies in the Psychology of Sex. Random House, New York.
36. Emmens, C. W. 1941. *J. Endocrinol.* **2,** 368–379.
37. Escamilla, R. F., and Lisser, H. 1941. *J. Clin. Endocrinol.* **1,** 633–642.
38. Felizet, G., and Branca, A. 1902. *J. anat. physiol.* **38,** 329.
39. Foss, G. L. 1938. *Lancet* **2,** 1284–1287.
40. Foss, G. L. 1939. *Brit. Med. J.* **2,** 11–12.
41. Frank, R. T., and Salmon, U. J. 1936. *Proc. Soc. Exptl. Biol. Med.* **34,** 804–805.

42. Fraser, R. W., Forbes, A. P., Albright, F., Sulkowitch, H., and Reifenstein, E. C , Jr. 1941. *J. Clin. Endocrinol.* **1**(3), 234–256.
43. Freud, S. 1924. Collected Papers, Vol. II. International Psychoanalytical Press, London.
44. Fruin, R. L. 1945. *Military Surgeon* **97,** 365–369.
45. Gallagher, T. F., Peterson, D. G., Dorfman, R. I., Kenyon, A. T., and Koch, F. C. 1937. *J. Clin. Investigation* **16,** 695–703.
46. Glass, S. J., Deuel, H. J., Jr., and Wright, C. A. 1940. *Endocrinology* **26,** 590–594.
47. Glass, S. J., and Johnson, R. H. 1944. *J. Clin. Endocrinol.* **4,** 540–544.
48. Glass, S. J., and McKennon, B. J. 1937. *Western J. Surg. Obstet. Gynecol.* **45,** 467–473.
49. Hamburger, C. 1931. *Ugeskrift. Laeger* **93,** 27–30.
50. Hamilton, J. B. 1937. *Endocrinology* **21,** 744–749.
51. Hamilton, J. B. 1941. *J. Am. Med. Assoc.* **116,** 1903–1908.
52. Hamilton, J. B., Catchpole, H. R., and Hawke, C. C. 1945. *J. Clin. Endocrinol.* **5,** 203–209.
53. Hamilton, J. B., Deming, C. L., and Allen, E. 1936. *Proc. Soc. Exptl. Biol. Med.* **34,** 193–194.
54. Hamilton, J. B., and Hubert, G. R. 1938. *Proc. Soc. Exptl. Biol. Med.* **39,** 3 4.
55. Hamm, L. 1942. *J. Clin. Endocrinol.* **2,** 325–328.
56. Harding, F. E. 1943. *J. Pediat.* **23,** 451–462.
57. Harding, F. E., and Fraser, A. V. 1942. *Urol. and Cutaneous Rev.* **46,** 575–580.
58. Heckel, N. J. 1939. *Proc. Soc. Exptl. Biol. Med.* **40,** 658–659.
59. Heckel, N. J. 1940. *J. Urol.* **43,** 286–308.
60. Heckel, N. J. 1941. *Trans. Am. Assoc. Genito-Urin. Surgeons.* **34,** 237–247.
61. Heller, C. G. (Detroit Group) 1944. *Macy Conf. Metabol. Aspects Convalescence* **7,** 60–68.
62. Heller, C. G., and Chandler, R. E. 1942. *J. Clin. Endocrinol.* **2,** 252–253.
63. Heller, C. G., and Heller, E. J. 1939. *Endocrinology* **24,** 319–325.
64. Heller, C. G., and Myers, G. B. 1944. *J. Am. Med. Assoc.* **126,** 472–477.
65. Heller, C. G., and Nelson, W. O. 1944. *Proc. Central Soc. Clin. Research* **17,** 74.
66. Heller, C. G., and Nelson, W. O. 1945a. *J. Clin. Endocrinol.* **5,** 1–12.
67. Heller, C. G., and Nelson, W. O. 1945b. *J. Clin. Endocrinol.* **5,** 27–33.
68. Heller, C. G., and Nelson, W. O. 1946. *Proc. Am. Soc. Clin. Investigation* **38,** 4.
69. Heller, C. G., Nelson, W. O., and Roth, A. A. 1943. *J. Clin. Endocrinol.* **3,** 573–588.
70. Heller, E. J., Heller, C. G., and Sevringhaus, E. L. 1941. *Endocrinology* **29,** 1–7.
71. Henry, G. W. 1934. *Psychiat. Quart.* **8,** 243–264.
72. Hirschfeld, M. 1940. Sexual Pathology. Emerson Books, New York.
73. Howard, J. E. 1941. *Bull. N. Y. Acad. Med.* **17,** 519–531.
74. Howard, J. E., Wilkins, L., and Fleischmann, W. F. 1942. *Trans. Assoc. Am. Phys.* **57,** 212–215.
75. Huggins, C., and Hodges, C. 1941. *Cancer Research* **1,** 293–297.
76. Huhner, M. 1939. *Med. Record* **149,** 366–370.
77. Hurxthal, L. M., and Wilson, W. H. 1944. *Lahey Clin. Bull.* **3,** 237–243.
78. Jaffe, I., and Brockway, G. 1942. *J. Clin. Endocrinol.* **2,** 189–192.
79. Johnson, W. W. 1939. *J. Am. Med. Assoc.* **113,** 25–27.
80. Jungck, E. C., Maddock, W. O., and Heller, C. G. 1947. *J. Clin. Endocrinol.* **7,** 1.

81. Kearns, W. M. 1941a. *J. Clin. Endocrinol.* **1,** 126–130.
82. Kearns, W. M. 1941b. *Wisconsin Med. J.* **40,** 927–931.
83. Kenyon, A. T.. 1938. *Endocrinology* **23,** 121–134.
84. Kenyon, A. T. 1941. *New Engl. J. Med.* **225,** 714–719.
85. Kenyon, A. T., Gallagher, T. F., Peterson, D. G., Dorfman, R. I., and Koch, F. C. 1937. *J. Clin. Investigation* **16,** 705–717.
86. Kenyon, A. T., Knowlton, K., Sandiford, I., Koch, F. C., and Lotwin, G. 1940. *Endocrinology* **26,** 26–45.
87. Kinsey, A. C. 1941. *J. Clin. Endocrinol.* **1,** 424–428.
88. Klinefelter, H. F., Jr. Albright, F., and Griswold, G. C. 1943. *J. Clin. Endocrinol.* **3,** 529–544.
89. Klinefelter, H. F., Jr. Reifenstein, E. C., Jr., and Albright, F. 1942. *J. Clin. Endocrinol.* **2,** 615–627.
90. Kochakian, C. D. 1946. *Vitamins & Hormones* **4,** 255–310.
91. Krafft-Ebing, R. 1892. Psychopathia Sexualis. F. A. Davis, Philadelphia.
92. Lacassagne, A. 1933. *Compt. rend. soc. biol.* **113,** 590–592.
93. Lesser, M. A. 1942. *New Engl. J. Med.* **226,** 51–54.
94. Lesser, M. A. 1943. *New Engl. J. Med.* **228,** 185–188.
95. Lesser, M. A. 1946. *J. Clin. Endocrinol.* **6,** 549–557.
96. Levine, E. B., and Sellers, A. L. 1946. *Am. J. Med. Sci.* **212,** 7–11.
97. Levine, S. A., and Likoff, W. B. 1943. *New Engl. J. Med.* **229,** 770–772.
98. Lippross, O. 1938. *Münch. med. Wochschr.* **85,** 1668–1672.
99. Lisser, H., and Curtis, L. E. 1943. *J. Clin. Endocrinol.* **3,** 389–399.
100. Lisser, H., Escamilla, R. F., and Curtis, L. E. 1942. *J. Clin. Endocrinol.* **2,** 351–360.
101. Lurie, L. A. 1944. *Am. J. Med. Sci.* **208,** 176–186.
102. McCullagh, E. P. 1940. *Cleveland Clin. Quart.* **7,** 226–230.
103. McCullagh, E. P., and McGurl, F. J. 1939. *J. Urol.* **42,** 1265–1273.
104. McCullagh, E. P., and McGurl, F. J. 1940. *Endocrinology* **26,** 377–384.
105. McCullagh, E. P., and Rossmiller, H. R. 1941. *J. Clin. Endocrinol.* **1,** 496–502.
106. McCutcheon, A. B. 1938. *Med. J. Australia* **1,** 654–657.
107. McGavack, T. H. 1943. *J. Clin. Endocrinol.* **3,** 71–80.
108. Markham, M. J. 1941. *Urol. and Cutaneous Rev.* **45,** 35–37.
109. Miescher, K., and Tschopp, E. 1938. *Schweiz. med. Wochschr.* **68,** 1258.
110. Miller, N. E., Hubert, G., and Hamilton, J. B. 1938. *Proc. Soc. Exptl. Biol. Med.* **38,** 538–540.
111. Mimpriss, T. W. 1945. *Proc. Roy. Soc. Med.* **38,** 507–508.
112. Moore, C. R., Lamar, V. K., and Beck, N. 1938. *J. Am. Med. Assoc.* **111,** 11–14.
113. Moore, R. A., and McLellan, A. M. 1938. *J. Urol.* **4,** 641–657.
114. Muellner, S. R., and Hamilton, J. B. 1944. *J. Urol.* **52,** 139–148.
115. Myerson, A., and Neustadt, R. 1942a. *Clinics* **1,** 932–957.
116. Myerson, A., and Neustadt, R. 1942b. *J. Mt. Sinai Hosp.* **9,** 668–678.
117. Nelson, W. O. 1940. *Am. J. Physiol.* **129,** 430.
118. Nelson, W. O. 1946. Unpublished data.
119. Nelson, W. O., and Heller, C. G. 1945. *J. Clin. Endocrinol.* **5,** 13–26.
120. Neustadt, R., and Myerson, A. 1940. *Am. J. Psychiat.* **97,** 524–551.
121. Oberndorfer, S. 1930. *Jahreskurse ärztl. Fortbild.* **21,** 19–29.
122. Österreicher, W. 1934. *Klin. Wochschr.* **13,** 1019–1022.
123. Owen, S. E., and Cutler, M. 1936. *Am. J. Cancer* **27,** 308–315.

124. Pace, J. M. 1935. *Proc. Staff Meetings Mayo Clinic* **10,** 726–730.
125. Pullen, R. L., Wilson, J. A., Hamblen, E. C., and Cuyler, W. K. 1942a. *J. Clin. Endocrinol.* **2,** 577–587.
126. Pullen, R. L., Wilson, J. A., Hamblen, E. C., and Cuyler, W. K. 1942b. *J. Clin. Endocrinol.* **2,** 655–663.
127. Pullen, R. L., Wilson, J. A., Hamblen, E. C., and Cuyler, W. K. 1942c. *J. Clin. Endocrinol.* **2,** 730–736.
128. Rawlings, L. B. 1908. *Practitioner* **81,** 250–257.
129. Rea, C. E. 1939. *Arch. Surg.* **38,** 1054–1107.
130. Rea, C. E. 1942. *Arch. Surg.* **44,** 27–34.
131. Reed, W. A., and McMillan, T. E. 1941. *New Orleans Med. Surg. J.* **93,** 634–637.
132. Reifenstein, E. C., Jr. 1946. *Ann. Meeting American Federation for Clinical Research.*
133. Rennie, T. A. C., Vest, S. A., Jr., and Howard, J. E. 1939. *Southern Med. J.* **32,** 1004–1007.
134. Riches, S. W. 1938. *Practitioner* **140,** 60–67.
135. Riseman, J. E. F. 1943. *New Engl. J. Med.* **229,** 670–680.
136. Rosenzweig, S., and Hoskins, R. G. 1941. *Psychosomat. Med.* **3,** 87–89.
137. Rubinstein, H. S. 1938. *J. Am. Med. Assoc.* **111,** 1818–1821.
138. Rubinstein, H. S. 1942. *J. Clin. Endocrinol.* **2,** 519–526.
139. Rubinstein, H. S., and Kurland, A. A. 1939. *Southern Med. J.* **32,** 499–503.
140. Saethre, H. 1935. *Klin. Wochschr.* **14,** 376–378.
141. Sand, K., and Okkels, H. 1938. *Endokrinologie* **19,** 369–379.
142. Scott, W. W., and Vermeulen, C. 1942. *J. Clin. Endocrinol.* **2,** 450–456.
143. Sevringhaus, E. L., and Chornyak, J. 1945. *Psychosomat. Med.* **7,** 302–310.
144. Sigler, L. H., and Tulgan, J. 1943. *N. Y. State Med. J.* **43,** 1424–1428.
145. Smith, P. E. 1944. *Yale J. Biol. Med.* **17,** 281–287.
146. Smith, P. H., Albright, F., and Dodge, E. 1943. *J. Lab. Clin. Med.* **28,** 1761–1766.
147. Smith, R. E. 1939. *Arch. Diseases Childhood* **14,** 1–13.
148. Soifer, S. 1941. *Urol. and Cutaneous Rev.* **45,** 582–584.
149. Southam, A. H., and Cooper, E. R. A. 1927. *Lancet* **1,** 805–811.
150. Spence, A. W. 1940a. *Brit. Med. J.* **2,** 411–412.
151. Spence, A. W. 1940b. *Quart. J. Med.* **9,** 309–321.
152. Spence, A. W. 1942. *Brit. Med. J.* **1,** 668.
153. Stekel, W. 1933. The Homosexual Neurosis. Phys. & Surg. Book Co., Brooklyn.
154. Strohm, J. G., Edelson, Z. C., and Merryman, G. H. 1938. *Urol. and Cutaneous Rev.* **42,** 510–511.
155. Strong, G. F., and Wallace, A. W. 1944. *Can. Med. Assoc. J.* **50,** 30–33.
156. Tager, B. N., and Shelton, E. K. 1941. *J. Clin. Endocrinol.* **1,** 131–133.
157. Talbot, N. B., and Butler, A. M. 1942. *J. Clin. Endocrinol.* **2,** 724–729.
157a. Thayer, S. A. 1946. *Vitamins & Hormones* **4,** 311–361.
158. Thomas, H. B., and Hill, R. T. 1940. *Endocrinology* **26,** 953–954.
159. Trasoff, A. 1940. *J. Lab. Clin. Med.* **25,** 377–383.
160. Varney, R. F., and Koch, F. C. 1942. *Endocrinology* **30,** 399–407.
161. Vest, S. A., and Barelare, B. 1943. *Clinics* **1,** 1216–1265.
162. Vest, S. A., and Howard, J. E. 1939. *J. Am. Med. Assoc.* **113,** 1869–1872.
163. Von Capellen, D. 1936. *Brit. J. Urol.* **8,** 45–49.
164. Wagenseil, F. 1933. *Z. Morphol. Anthropol.* **32,** 415–468.

165. Waldman, S. 1945. *J. Clin. Endocrinol.* **5**, 305–317.
166. Walker, T. C. 1940. *Med. Record Ann.* **34**, 667.
167. Walker, T. C. 1942. *J. Clin. Endocrinol.* **2**, 560–568.
168. Wangensteen, O. H. 1932. *Surg. Gynecol. Obstet.* **54**, 219–231.
169. Webster, B. J. 1938. *J. Pediat.* **13**, 847–858.
170. Werner, A. A. 1939. *J. Am. Med. Assoc.* **112**, 1441–1443.
171. Werner, S. C. 1943. *J. Clin. Investigation* **22**, 395–401.
172. Wright, C. A. 1935. *Med. Record* **142**, 407–410.
173. Wright, C. A. 1938. *Med. Record* **147**, 449–452.
174. Wright, C. A. 1939. *Med. Record* **149**, 399–402.
175. Wright, C. A. 1941. *Med. Record* **154**, 60–61.
176. Zeithaml, C. E. 1938. *Clin. Bull. Univ. Hosp. Cleveland* **2**, 67–70.
177. Zelson, C., and Steinitz, E. 1939. *J. Pediat.* **15**, 522–534.
178. Zelson, C., and Steinitz, E. 1940. *J. Pediat.* **17**, 315–321.
179. Zondek, B., and Sulman, F. 1939. *Proc. Soc. Exptl. Biol. Med.* **40**, 633–637.
180. Zuckerman, S. 1936. *Lancet* **2**, 1259–1262.
181. Zuckerman, S., and Parkes, A. S. 1936. *Lancet* **1**, 242–247.

Author Index

Names in parentheses indicate senior authors of the references and are included to assist in locating references where a particular name is not on a given page.

Example: Abarbanel, A. R., 329 (see Sturgis) means that Sturgis *et al.* will be mentioned on page 329, the *et al.* accounting for Abarbanel. This article can be located under Sturgis in the list of references. Numbers in italics refer to the pages on which references are listed in bibliographies at the end of each article.

A

Abarbanel, A. R., 321, 329 (see Sturgis), 342, 344, 345 (see Sturgis), 355, 357, 359 (see Silberman), 362, 370, 374, 381 (see Feldman), *383*, *385*, *389*, *390*
Abel, S., 332, 335, 350, 362, *383*
Abels, J. C., 52 (see Klopp), *88*
Acuna, J., 265 (see Lipschütz), *270*
Adair, F. E., 333, 335, 362, 372, *383*, *386*
Adams, A. E., 282, 294, *307*
Addison, T., 141
Adler, A., 420, *428*
Adlersberg, D., 50, 58, *85*, 135, *157*
Agid, R., 96, 111, *116*
Ahmad, B., 60, *85*
Akel, J., 248, *270*
Albert, A., 295, *313*, 316
Albert, S., 266, *267*
Albright, F., 225 (see de las Balze), *227*, 335 (see Kinsell), 337 (see Reifenstein), 338, 351, 353, 375–377, *383*, *387*, *388*, *390*, 396–398, 401 (see Fraser; Klinefelter), 407 (see Fraser), 408 (see Fraser), 409, *429–431*
Alexander, B., 51, 67, *85*
Allanson, M., 250, *267*
Allen, B. M., 286, *307*
Allen, E., 339, *383*, 425, *429*
de Allende, I. L. C., 331, *384*
Almquist, H. J., 61, 79, *85*, 164 (see Cravens), 169 (see Cravens), *172*
Alpers, B. J., 239, 244, *270*
Ambrose, A. M., 60 (see Wilson), *92*
Amill, L. A., 145, 146, *157*
d'Amour, F. E., 243, 247, 248, *269*, *271*
Anderson, E., 284 (see Morton), *312*, 374, *383*
Anderson, E. M., 215 (see Collip), *228*
Anderson, G., 49 (see Hawley), 63 (see Hawley), *87*
Anderson, G. W., 303, *307*
Anderson, R. C., 303, *308*
Andervont, H. B., 372, *388*
Andrews, F. N., 283, 285, 286, 297, 299, 302, *307*, *313*
Andrews, G. A., 49 (see Bethell), 77 (see Bethell), *85*, 151 (see Bethell), 155 (see Bethell), *157*
Andrews, J. S., 45, 83, *85*
Angier, R. B., 119, 121, *157*
Anglem, T. J., 305 (see Williams), *314*
Applegarth, A. P., 212, *227*
Aranow, H., Jr., 277–279, 285, *307*, *309*
Archer, W., 169 (23, 24), *172*
Arens, J. F., 27, 28, 34, *36*, *38*
Arndt, H., 422, *428*
Arnold, A., 45 (8), 64, *85*, 164 (1), *171*
Arnold, R. T., 28 (see Fuson), *36*
Aron, H., 225, 226, *230*
Aronson, V., 216 (see Reiss), *230*
Arrighi, L., 348 (see Salaber), *389*
Ashburn, L. L., 60 (see Daft), 74 (see Daft), *86*, 297, 298, 300, *307*, *308*
Asling, C. W., 213 (see Becks), *227*
Asmundson, V. S., 164 (6), 166 (see Bird), 170 (see Bird; Lepkovsky), *172*, *173*
Astwood, E. B., 216, *227*, 259, 261, *267*, 275, 277–279, 281–290, 293, 294, 298–301, 303–305, 307, *307–310*, *312*, 316
Atkin, L., 45 (10, 11, 235), 46, 52 (see Light), 72, *85*, *89*, *91*, 99, *116*
Atkins, H. J. B., 367, *383*
Aub, J. C., 294, *313*, 394, *428*
Audit, M. J., 367, *383*
Axelrod, H. E., 73 (see Morgan), *89*
Axelson, G. J., 327 (see Hamblen), 329 (see Hamblen), *386*

B

Babcock, S. H., 72 (see Spies), *91*
Babcock, S. H., Jr., 183 (see Daft), *194*
Bacharach, A. L., 1, 2, *36*
Bachman, C., 237, 238, *267*
Badgett, C. D., 83, *85*
Bailey, G. F., 60 (see Wilson), *92*
Baird, F. D., 164(2), *171*

Baker, R. F., 287, 292, 295, *309*
Balin, J., 216, *230*
Balkanyi, J., 96, 111, *116*
Banerjee, S., 189, *194*
Banting, F. G., 225, *227*
Barelare, B., 394, *431*
Barker, D., 169 (23, 24), *172*
Barker, M. H., 275, *308*
Barker, S. B., 284, 305, *308*
Barker, W. H., 124, *157*
Barnes, B., 381, *384*
Barnes, B. O., 244, *267*
Barr, D. P., 285, *308*
Barrett, R., 41, 67, 77 (see Najjar), *89*
Barron, E. S. G., 115, *116*
Bartelmez, G. W., 339, *383*
Bartels, E. C., 305, *308*, 316
Bartz, Q. R., 199 (see Evans), 200 (see Evans), 202 (see Evans), *228*
Basinski, D. H., 64, *85*
Bates, R. W., 215, 220, *227*
Batty, J. W., 35, *38*
Bauer, J. L., 144, 146, 149, *157*
Bauernfeind, J. C., 45, *85*, 164 (3), *172*
Baumann, C. A., 59 (see Lease), 73, 79 (see Jacobi), *88*, *89*, 185–188, *194*
Baumann, E. J., 275, 277, 287, 296, 298–300, *308*, *311*
Baxter, J. G., 32, 33, 35 (see Cawley), *38*, 44, *85*
Beach, F. A., 420, *428*
Beadle, G. W., 45 (107), *87*
Beall, D., 235, *267*, *268*
Beard, H. H., 190, *194*
Bearse, G. E., 164 (4), *172*
Beato, V., 344, *383*
Beatson, G. T., 371, *383*
Beck, N., 412, *430*
Beckers, W., 351, *383*
Becks, H., 200, 208 (see Simpson), 213, 221, 222, *227*, *229*, *230*
Béclère, C., 344, 346, *383*
Beecham, C. T., 350, *383*
Beilly, J. S., 370, *383*
Bell, G. H., 238, *268*
Bender, X., 367, *383*
Benedek, T., 361, *383*
Benesch, R., 51, 71, *85*, *86*
Bennett, L. L., 212, 215, 222 (see Gordan), 225, *227*, *228*
Berblinger, W., 248, *268*
Berg, B., 357, *387*
Bergami, G., 94, 95, *116*
Bergeim, O., 50 (see Cornbleet; Kirch), *86*, *88*
Berlind, M., 322, 327, 332, 348, 359, 362, 370, *383*
Bernstien, J., 381, *383*
Berry, L. J., 121, 122, *157*
Berryman, G. H., 51 (see Denko), 72 (see Denko), 74 (see Denko), 75 (see Denko), 77 (see Denko), *86*
Bessey, O., 43, *85*
Bessey, O. A., 169, *173*, 177, *195*
Best, C. H., 78, *85*
Bethell, F. H., 49, 77, *85*, 147 (see Isaacs), 148 (see Isaacs), 151, 155, *157*, *159*
Bethke, R. M., 164 (5,52,53) (see Cravens), 165 (see Record), 167, 169 (see Cravens), *172*, *173*
Beutner, R., 293, *309*
Bevelander, G., 283, *308*
Bhagvat, K., 65, *85*
Bialet-Laprida, Z., 244, 251, *268*
Bickers, W., 331, 352, *383*
Bicknell, F., 111, 114 (see Chamberlain), *116*
Bielschowsky, F., 279, 282, 306, *308*
Bierbaum, O. S., 122 (see Moore), 123 (see Moore), 125 (see Moore), 133 (see Moore), 141 (see Moore), 142 (see Moore), 143 (see Moore), 144 (see Moore), 145 (see Moore), 146 (see Moore), 148 (see Moore), 149 (see Moore), *159*
Bierring, E., 212
Binet, L., 94–96, *116*
Binkley, S. B., 120, 151, *157*, *160*
Birch, T. W., 73, *85*
Bird, F. H., 164 (6), 166, 170, *172*, *173*
Bird, H. R., 165, 166, 168, 171, *172*, *173*
Bird, O. D., 45 (23), 47, 49 (see Bethell), 76 (see Campbell), 77 (see Bethell), *85*, *86*, 120, 151, 155 (see Bethell), *157*, *160*
Birnberg, C. H., 359, 370, *384*, *387*
Bishop, B. W. F., 344, *384*
Bishop, P. M. F., 417, *428*
Biskind, G. R., 324, *384*, 412, *428*

Bissell, A., 277–279, 281, 282, 285–288, 294, 303, 304, *308*, *314*

Bissell, G. W., 280, 284, 285, 295–297, 298 (see Williams), 305, *314*, 376 (see Williams), 378 (see Williams), *391*

Black, A., 188, *194*

Black, D. A. K., 132, *157*

Black, E. F. E., 344, 346, *384*

Black, S., 61, *85*

Bliss, C. I., 49, *85*

Blivaiss, B. B., 286, *309*

Bloch, E., 1, 2, 27 (see Sobotka), *38*

Bloch, I., 419, *428*

Bloom, E. S., 120 (see Binkley; Bird; Pfiffner), 121 (see Pfiffner), 151 (see Binkley; Bird; Pfiffner), *157*, *160*

Bloomberg, E., 338, 375 (see Albright), 376 (see Albright), *383*

Boehme, W., 83 (see Huber), *87*

Bogatin, B., 135, *157*

Bogert, M. T., 1, *36*

Bohonos, N., 120 (see Hutchings), 121 (see Hutchings), 122 (see Hutchings), *159*

Bolan, T. R., 32 (see Morrell), *38*

Bolger, W. P., 332, 373, *384*

Bolton, W., 164 (10), *172*

Bompard, E., 326 (see Laroche), 328 (see Laroche), 359 (see Laroche), 360 (see Laroche), *387*

Bond, A. C., Jr., 35, *38*

Bonnell, R. W., 422, *428*

Bonser, G. M., 240, *268*

Bonsnes, R. W., 198, *230*

Booth, R. G., 60, *85*

Boothe, J. H., 119 (see Angier), 121 (see Angier), *157*

Boquet-Jesensky, M., 380, *388*

Borchardt, H., 235, 242, 260, *268–270*, 395, *428*

Borden, R. A., 49, 63, *86*

Borland, J. R., 276, *308*

Borsook, H., 63, *85*

Boruff, C. S., 45 (see Bauernfeind), *85*

Bos, L. H., 327, *386*

Boucher, R. V., 170 (see Patrick), *173*

Boyd, H. M., 45 (see Andrews), *85*

Bradbury, J. T., 297, *310*

Bradley, H. T., 281, *312*

Brahn, B., 421, *428*

Branca, A., 418, *428*

Brazie, D., 164 (18), *172*

Brenner, J., 54, *85*

Brew, W., 43, 44, *85*

Briggs, A. P., 70 (see Singal), 74 (see Sydenstricker), *91*, *92*

Briggs, G. M., Jr., 155 (see Luckey), *159*, 164 (14–16, 26), 167, 168, 169 (12), 170, 171, *172*, 286, *308*

Brill, A. A, 420, *428*

Britton, S. W., 225, *228*

Brockway, G., 418, *429*

Brovsin, I., 252, 267, *270*

Brown, G. L., 109, *116*

Brown, R. A., 44 (see Ewing), 49 (see Bethell), 76 (see Campbell), 77 (see Bethell), *86*, 120 (see Binkley, Pfiffner), 151 (see Bethell; Binkley), 155 (see Bethell), *157*, *160*, 164 (17), 168 (see Campbell); 170 (see Campbell), *172*

Browne, J. S. L., 225, *230*, 374, *390*, 414, *428*

Bruce, W. F., 168 (see Scott), 170 (see Scott), 171 (see Scott), *173*

Brücke, F. v., 96, *116*

Bruger, M., 287, *311*

Bruzzone, S., 265 (see Lipschütz), *270*

Bryan, A. H., 374 (see Kenyon), 377 (see Kenyon), *386*

Bryan, W. L., 190, 191, *194*

Bryant, D., 335 (see Kinsell), *387*

Bühler, F., 380, *384*

Bugbee, E. P., 243, *268*

Bulbring, E., 199, *227*

Buraway, A., 35, *38*

Burch, J. C., 250, *268*

Burchenal, J. H., 122 (see Castle), 126 (see Castle), 150 (see Castle), *157*

Burcura, C. J., 342, *384*

Burkholder, P. R., 45 (30), 47, *85*

Burns, R. K., 241, 242, *268*

Burrill, M. W., 241, 242 (see Greene), 266 (see Greene), *269*, 321 (see Greene), 323 (see Greene), *386*

Burrows, H., 234–236, 239, 249, 254, 255, 258, 260, 263, *268*, 425, *428*

Burtner, E., 219, 220, *227*

Burton, B. W., 62, *86*

Buschke, W., 286, 296, *309*

Butler, A. M., 322, 377, *390*, 396, *431*
Butturini, L., 106, *116*
Byerrum, R. V., 300, *308*
Byron, C. S., 322, *384*, 412, *428*
Bywater, W. G., 303, *308*, *312*

C

Cahill, W. M., 49, 64, *85*
Calabro, Q., 94, 95, *116*
Caldwell, E., 43, *91*
Caldwell, M. H., 123 (see Spies), *160*
Calkins, D. G., 120 (see Binkley; Pfiffner), 121 (see Pfiffner), 151 (see Binkley, Pfiffner), *157*, *160*
Callow, N. H., 235, 266, *268*, 328, 337, *384*, 396, 397, *428*
Callow, R. K., 235 (see Callow, N. H.), *268*, 328 (see Callow, N. H.), 337, *384*, 396, 397, *428*
Calvo, J., 316
Campbell, B. K., 18, *36*
Campbell, C. J., 76, *86*, 120 (see Binkley, Pfiffner), 151 (see Binkley), *157*, *160*, 164 (17), 168, 170, *172*
Campbell, D., 293, 301, 302, *308*
Campbell, K. N., 18, 19, *36*
Campbell, M. A., 4 (see Milas), 9 (see Milas), 15 (see Milas), 16 (see Milas), 18 (see Milas), 19 (see Milas), 22 (see Milas), *37*, *38*
Cantarow, A., 278, 291–293, 297, 298, 300–302, 305, *312*
Capitain, 344, 345, 359, 367, *384*
Carlson, W. E., 65 (see Green), *87*
Carmichael, H. T., 419, *428*
Carroll, E., 337 (see Reifenstein), *388*
Carpenter, L. E., 45, *92*
Carruthers, L. B., 154, *157*
Carter, A. C., 330, 358, 376, *384*, *389*
Carter, G. S., 305, *308*
Carver, J. S., 164 (18), *172*
Caspersson, T., 99, *116*
Castle, W. B., 122, 124, 126, 132, 133, 139–141, 143, 144, 146, 149, 150, 152, *157*, *159*, *161*
Catchpole, H. R., 198, *231*, 398, 406 (see Hamilton), 408, 409, *428*, *429*
Cawley, J. D., 6, 35, *38*
Cerecedo, L. R., 185, 189, 191–193, *194*, *195*
Chagas, C., 288, *308*
Chaikoff, I. L., 284, 285, 287, 288 (see Wolff), 289–291, 293 (see Taurog), 303, *309*, *311–314*
Chamberlain, W. P., 114, *116*
Chan, H., 191 (see Snell), *195*
Chandler, R. E., 358 (see Heller), *386*, 397, *429*
Chang, M.-C., 241, *268*
Chanutin, A., 315
Chapman, A., 284, *308*
Charipper, H. A., 277, 279, 281–287, 288 (see Keston), 289, 294–299, 305,307, *309–312*, *314*
Charny, C. W., 398, 405, 411, 416, *428*
Chase, J. H., 226, *228*
Cheldelin, V. H., 45 (226), *91*
Chen, K. K., 303, *308*
Cherrington, M. E., 133, *157*
Chesley, L. C., 301, 302, *308*
Chesney, A. M., 275, 287, *308*, *314*
Chiapponi, L., 250, *271*
Child, G. P., 352, *384*, *391*
Chiray, M., 380, *384*
Chornyak, J., 419, 421, *431*
Chow, B. F., 198 (see Greep; Shedlovsky), *229*, *230*
Christ, R. E., 1, *36*
Christensen, B. G., 284, *308*
Christensen, H. N., 302, *308*
Church, C. F., 67, *92*
Ciereszko, L. S., 198, *227*
Cinberg, B. L., 354, 381, *384*
Ciner, L. F., 370, *386*
Cipra, A., 275, *311*
Clahr, J., 371, *384*
Clark, G. W., 126, *157*
Clark, P. F., 154 (see Waisman), *161*
Clark, P. J., 240, *270*
Clarke, M. F., 46, *89*
Clausen, S. W., 50, 58, *86*
Clawson, T. A., 275, *308*, *314*
Clayton, M. M., 49, 63, *86*
Cline, J. K., 123 (see Spies), 154 (see Spies), *160*
Clisby, K. H., 276–278, 298, *313*
Clute, H. M., 302, 305, *314*
Clutterbuck, P. W., 125, *161*
Coffman, J. R., 328 (see Hoskins), *386*
Coggi, C., 344, *384*

Cohen, E. J., 331, *389*
Cohen-Solal, G., 265, *268*
Cohn, E. J., 201 (see Fevold), *228*
Coindet, 274
Cole, H. H., 212, *229*
Collins, M. B., 297, 298, *312*
Collip, J. B., 202, 215, 221, *228*, *230*, 237, 238, 246, 260, *267*, *268*, *271*
Collord, J., 49, 50, 69, 72 (see Parsons), 74 (see Parsons), *90*
Colombo, E. O., 370, *388*
Conger, T. W., 16 (see Hanze), 20 (see Hanze), *36*
Cook, B. B., 68 (see Morgan), *89*
Cooke, H. G., Jr., 28 (see Fuson), *36*
Cooper, E. R. A., 418, *431*
Cooperman, J. M., 121, 122, *157*
Cope, O., 288 (see Rawson), *313*
Corey, E. L., 225, *228*
Cornbleet, T., 50, *86*, *88*, 381, *384*
Corner, G. W., 331, 339, *384*, *389*
Cornish, R. E., 201, *228*
Corsaro, J., 322, 370, *384*
Cortell, R. E., 280, 288, 289, 305 (see Rawson), *313*
Cosulich, D. B., 119 (see Angier), 121 (see Angier), *157*
Cottier, 111
Couceiro, A., 288, *308*
Couch, J. R., 166, *172*
Coulson, C. A., 32, *36*
Coulson, R. A., 51, 71, *86*
Courrier, R., 235, 238, 253, 255, 265, *268*
Coward, K. H., 67 (see Cross), *86*
Cowgill, G. R., 40, 64 (see Himwich), *87*
Cowie, A. T., 324, *384*
Cracas, L. J., 52 (see Light), *89*
Cramer, W., 248, 249, 260, 262, 263, *268*
Crampton, E. W., 62, *86*
Crane, A. R., 315
Cravens, W. W., 164, 169, *172*
Creevy, C. D., 419, 426, *428*
Cristol, S. J., 33, *36*, *38*
Crook, E. M., 63, *86*
Crooke, A. C., 250, *268*
Cross, M. C. A., 67, *86*
Cunha, T. J., 74, *89*
Cunningham, R. S., 250, *268*
Curtis, A. C., 49, 57, *86*
Curtis, L. E., 323, *387*, 412, *430*
Cuthbertson, D. P., 238, *268*
Cutler, M., 425, *430*
Cuyler, W. K., 327 (see Hamblen), 329 (see Hamblen), *386*, 394 (see Pullen), *431*
Cymerman, J., 2, *36*
Czaczkes, J. W., 68, *86*

D

Daft, F. S., 60, 74, 76, *86*, 121, 122 (see Watson), 135, 156, *157*, *159*, *161*, 183, *194*, 296–298, 300, *307–309*, *311*
Dale, H. H., 94, *116*
Dalsace, J., 370 (see Portes), *388*
Dalton, A. J., 279, 282, 295, 296, 299, 306, *308*, *309*
Dam, H., 168, *172*
Daniel, L. J., 76, *86*, 164 (56), *173*
Dann, W. J., 52 (see Kline), 69, *86*, *88*
Danner, M., 263, *268*
Danowski, T. S., 285, 301, *309*
Darby, W. J., 120, 122, 123, 125, 126 (see Langston), 127, 131–140, 143–146, 148, 149, 154 (see Day), *157–159*
Daron, G. L., 339, *384*
Davenport, H. W., 63 (see Boorsook), *85*
David, K., 251, *268*
Davidson, C. S., 122 (see Castle), 126 (see Castle), 150 (see Castle), *157*, 224, *228*
Davies, W. H., 2, 22, *36*
Davis, J. E., 156, *158*
Davis, M. V., 68, *86*
Davis, R. R., 32 (see Morrell), *38*
Davison, H. G., 68 (see Morgan), *89*
Dawson, A. B., 315
Day, P. L., 76 (see Totter), *92*, 120–122, 125, 126, 135 (see Mallory), 138, 140, 150, 151, 154, 155, *158*, *159*
Deakins, M. L., 375, *384*
Deanesly, R., 239, 244–247, 263, 266, *268*, *269*, 324, *384*, 413, *428*
De Caro, L., 106, *116*
De Eds, F., 60 (see Wilson), *92*
De la Balze, F. A., 225, *227*
del Castillo, E. B., 239, *269*, 339, 344, 348, *384*, *389*
Delfs, E., 296, *310*

De Marie, A., 282, 286, *311*
Deming, C. L., 425, *429*
Dempsey, E. W., 284, 290–292, 300, 307, *309*
Denk, W., 418, *428*
Denko, C. W., 51, 72, 74, 75, 77, *86*
Dennison, M., 236 (see Korenchevsky), 239, 252, 263, 264, 266, 267, *270*
De Nogales, C., 316
Denslow, C., 426, *428*
DeRitter, E., 49 (see Furter), 52 (see Furter), 83 (see Furter), *86*
De Robertis, E., 288, 290, 292, *308*, *309*
De Ronde, M., 334, *388*
Desclin, L., 250, *269*
Desmarest, 344, 345, 359, 367, *384*
Deuel, H. J., Jr., 32, *36*, 42, *86*, 419, 421, 422, *429*
Deutsch, H. F., 65, *86*
DeVaughn, N. M., 74 (see Sydenstricker), *92*
Devi, P., 65, *85*
De Vita, J., 239, *269*
DeWitt, J. B., 44, *86*
Dieke, S. H., 299, 301, *309*, 315
Dietrich, W. C., 293, *309*
van Dijk, G. M., *36*
Dingemanse, E., 202, *228*, 235, 242, 260, *268–270*, 395, 425, *428*
Di Palma, J. R., 296, 299, *309*, 338 (see Reynolds), *388*
Di Paola, G., 339, *384*
Dittmer, K., 46 (see du Vigneaud), *86*
Doan, C. A., 121 (see Saslaw), 122, 123, 143, 146, 149, *157*, *158*, *160*
Dobriner, K., 337, 375, *384*
Dock, W., *428*
Dodds, E. C., 234, 240, *269*
Dodge, E., 397, *431*
Döhrn, M., 238, 250, *269*
Doisy, E. A., 234 (see Huffman), 239, *270*, *272*
Dols, M. J. L., 166, *172*
Domm, L. V., 286, 297, *309*, *313*
Donaldson, E., 337 (see Reifenstein), *388*
Dorfam, F., 177 (see Jones), 182 (see Jones), 194
Dorfman, R. I., 235 (see Gallagher), *269*, 328, *384*, 396 (see Gallagher; Kenyon), 397 (see Gallagher), *429*, *430*
Dott, N. M., 200, *228*
Douay, E., 346, 367, *384*
Dougherty, T. F., 225, 226, *228*, *231*
Downs, W. G., Jr., 212, *228*
Doyle, W. L., 297, 300, *309*, 315
Drake, C. B., 417, *428*
Draper, J. W., 426, *428*
Drekter, L., 73 (see Rubin), *90*
Dreyer, N. B., 296, 299, *309*
Drill, V. A., 274, *309*
Drummond, J. C., 1 (see Heilbron), *36*
Dubnik, C. S., 177, 180, 185, *194*, 279, 282, 295, 296, 299, 306, *308*, *309*
Du Bois, K. P., 297, 300, *309*, 315
Duckman, S., 370, *384*
Duffy, P. V., 322, 370, *384*
Dunn, S. C., 153, *160*
Dunn, T. B., 177 (see Morris), *194*
Dunning, W. F., 266, *271*
Duntley, S. Q., 338 (see Edwards), *384*
Duret, M., 380 (see Chiray), *382*
Durgin, R. C., 164 (66), 165, *173*
Dutcher, R. A., 66, 68, *87*, 170 (see Patrick), *173*
Dutton, H. J., 60 (see Wilson), *92*
du Vigneaud, V., 46, *86*
Dziemian, A., 292, *309*

E

Eakin, R. E., 74 (see György), *87*
Eccles, W. M., 418, *428*
Edelson, Z. C., 425, *431*
Edgerton, R. O., 2, 4 (see Milas), 30, *37*
Edwards, E. A., 338, *384*
Edy, L. T., 19, *36*
Egger, K., 426, *428*
Eidelsberg, J., 413, *428*
Eijkman, C., 93, *116*
Einhauser, M., 64, *86*
Eldridge, M., 267, *270*
Elkin, M., 141, 144, *160*
Ellinger, P., 51, 71, *86*
Ellis, A., 419, 421, *428*
Ellis, H. H., 419, *428*
Elmby, A., 62, *86*
Elsom, K. O., 49 (see Reinhold), 64 (see Reinhold), *90*
Elvejhem, C. A., 45 (8), 46 (see Oleson), 47 (see Krehl; Sarma), 61 (see

Black), 64, 67 (see McIntire), 68–70, 71 (see Krehl), 73 (see Sarma), 74, 76, 83 (see Krehl), 84 (see Krehl), *85*, *86*, *88–91*, 121 (see Cooperman), 122 (see Cooperman), 140, 154 (see Waisman), 155 (see Luckey), *157*, *159*, *161*, 163 (see Luckey), 164 (1, 14–16, 26, 27, 43, 67), 167 (see Hegsted; Sarma), 168 (see Briggs; Hegsted; Luckey), 170 (see Briggs), 171 (see Briggs), *171–173*, 183 (see Mills), 189, *194*
Embleton, J., 67 (see Cross), *86*
Embree, N. D., 6, 15, 35 (see Cawley), *36*, *38*
Emery, F. E., 247, *270*
Emmens, C. W., 233, 235 (see Callow), 239, 265, *268*, *269*, *271*, 324, 328 (see Callow), 337, *384*, 396, 412, *428*
Emmerle, A., 15 (see van Eekelen), *36*
Emmett, A. D., 44 (see Ewing), 76 (see Campbell), *86*, 120 (see Binkley; Bird; Pfiffner), 151 (see Binkley; Bird), *157*, *160*, 164 (17), 168 (see Campbell), 170 (see Campbell), *172*
Endicott, K. M., 60 (see Daft), *86*, 296, 297, 298 (see Ashburn), 300, *307–309*, *311*
Engel, E. T., 243, *269*
Engel, R. W., 79, *86*, 114, *116*, 183, *195*
Engle, E. T., 277–279, 285, *307*, *309*, *310*, *313*, 339, 377, *388*
Engle, L. L., *390*
English, A., 145 (see Spies), 154 (see Frommeyer; Spies), *158*, *160*
Erdheim, J., 244, *269*
Escamilla, R. F., 322–324, *384*, *385*, *387*, 412, *428*, *430*
von Euler, H., 13, *36*
von Euw, J., 222 (see Reichstein), *230*
Evans, B. D. F., 125, 139, *161*
Evans, C. A., 65 (see Green), *87*
Evans, H. M., 64, *86*, 198 (see Fraenkel-Conrat; Li), 199–202, 204–209, 212 (see Fraenkel-Conrat; Marx; Simpson), 213 (see Becks), 215 (see Li), 216 (see Simpson), 218, 220 (see Li), 221, 222 (see Becks; Fraenkel-Conrat; Li), 224, 225 (see Ingle), *227–230*, 249 (see Koneff), *270*
Evans, J. S., 225 (see Ingle), *229*
Evans, R. D., 288, 305, *310*, *313*
Evelbauer, K., 367, *385*
Evenson, A., 76 (see Moore), *89*
Ewing, D. T., 44, *86*

F

Fabre, M., 346, *385*
Fahrenbach, M. J., 119 (see Angier), 121 (see Angier), *157*
Falk, E. A., 337, *388*
Farmer, F. A., 164 (56), *173*
Farrow, J. H., 372, *385*
Fartmann, B., 32, *38*
Fastier, F. N., 296, *309*
Fatzer, A. S., 49, 63, *92*
Favier, J., *36*
Feiman, D. T., 354, *385*
Feldberg, W., 109, *116*
Feldman, S., 381, *385*
Felizet, G., 418, *428*
Fels, E., 372, *385*
Ferrebee, J. W., 375 (see Deakins), *384*
Fevold, H. L., 201, *228*, 329, *385*
Field, H., Jr., 49, 64, 65 (see Melnick; Robinson), 82 (see Melnick), 84 (see Melnick), *86*, *89*, *90*
Finholt, A. E., 35, *38*
Finkelstein, G., 297, 298 (see Goldsmith), *309*, *310*
Firor, W., 339, *386*
Fischer, G. S., 44, *86*
Fishberg, E. H., 298, *309*
Fitzhugh, O. G., 315
Fleischer, A. J., 370, 374, *385*
Fleischmann, G., 51 (see Najjar), 52 (see Najjar), 68 (see Najjar), 71 (see Najjar), 74 (see Najjar), 77 (see Najjar), *89*
Fleischmann, W., 321–323, 377, *390*, *391*
Fleischmann, W. F., 412, *429*
Fletcher, F., 143, 145 (see Wilkinson), 146, 148, *161*
Foeste, A., 66 (see Parsons), *90*
Folley, S. J., 262, *269*, 324, 365, 370, *384*, *385*, *390*
Follis, R. H., 70 (see Wintrobe), *92*
Folsom, M. T., 49, 63, *86*
Foltz, L. M., 326, 381 (see Rothermich), *389*

Foote, E. C., 296, *310*
Foote, F. W., 365, 366, 371, *385*
Forbes, A. P., 337 (see Reifenstein), 377, *383*, *388*, 396, 398 (see Fraser), 401 (see Fraser), 407, 408 (see Fraser), *429*
Forbes, J. C., 286, 297, *311*
Forbes, T. R., 324, *385*
Ford, Z. W., 67, *92*
Forminge, P., 316
Foss, G. L., 329, 333, 335, 343, 359, 360, 367, *385*, 412, *428*
Foster, C., 177 (see Jones), 182 (see Jones), *194*
Foster, F. I., 338 (see Reynolds), *388*
Foulger, M. P. H., 275, *309*
Fourman, P., 132, *157*
Fox, H. J., 122 (see Castle), 126 (see Castle), 150 (see Castle), *157*
Foy, T. R., 45 (122), *88*
Fraenkel-Conrat, H., 198, 199, 201, 202, 212 (see Fraenkel-Conrat, J.), 222, *228*
Fraenkel-Conrat, J., 212, *228*
Frame, E. G., 303, 304, *314*
Frank, A. H., 260 (see Turner), 261, *272*
Frank, H. E., 152, *159*
Frank, R. T., 356, *385*, 408, *428*
Fraps, G. S., 50, 59, 60, *86*, 164 (62, 63), 166 (see Couch), *172*, *173*
Fraser, A. V., 413, *429*
Fraser, J., 200, *228*
Fraser, R. W., 396, 398, 401, 407, 408, *429*
Frazier, C. N., 243, 261, *269*
Freed, A. M., 66 (see Weswig), *92*
Freed, S. C., 321, 329, 332, 343–346, 354, 356, 357, *385*, *386*
Freeman, W., 362 (see Rubinstein), *389*
de Fremery, P., 247, 262, *269*, *272*
Freud, J., 200, 202, *228*, 251–253, 261, 266, *268*, *269*
Freud, S., 420, *429*
Frey, C. N., 45 (10, 11, 235), 46 (see Atkin; Schultz), 72 (see Atkin), *85*, *91*, 99, *116*
Friedemann, T. E., 51 (see Denko), 72 (see Denko), 74 (see Denko), 75 (see Denko), 77 (see Denko), *86*
Friedenwald, J. S., 286, 296, *309*
Friedgood, H. B., 375 (see Deakins), *384*
Friedman, A., 133, 145, 146, 147 (see Isaacs), 148 (see Isaacs), *159*
Fritz, J. C., 169 (23, 24), *172*
Frommeyer, W. B., Jr., 123 (see Spies), 132 (see Spies), 145 (see Spies), 154, *158*, *160*
Fruin, R. L., 417, *429*
Fry, E. G., 226 (see Sayers), *230*, 374 (see Long), *387*
Furter, M. F., 49, 52, 69, 83, *86*
Fuson, R. C., 1, 28, *36*

G

Gaarenstroom, J. H., 246, *269*
Gaddum, J. H., 95, *116*
Gaebler, O. H., 208, 209, 211, *228*
Gaines, J. A., 327 (see Geist; Salmon), 329, 333, 343 (see Geist; Salmon), 346 (see Salmon), *385*, *389*
Gairns, S., 225, *227*
Gallagher, T. F., 235, *269*, 395–397, *429*, *430*
Gardner, J., 49 (see Parsons), 50, 51, 69 (see Parsons), 72 (see Parsons), 74, *86*, *90*
Gardner, W. U., 236, 239, 247, 260, 261, 264, *269*, *272*
Gasche, P., 315
Gassner, F. X., 276, 287, *314*
Gehrke, M., 244, *271*
Geist, S. H., 327, 329, 331, 332 (see Salmon), 333 (see Gaines), 343, 345, 346, 351, 354, 357, 359, 360, 362, 367, *385*, *389*
Geomine, J., 316
Gerl, A. J., 285, 305, *312*, 316
Gersh, I., 287, 292, 295, *309*
Geschickter, C. F., 259, 261, *267*, 365, 369, *385*, *387*
Gibbs, 206
Gilberg, H., 66 (see Parsons), *90*
Gilbert, J., 251, *269*
Gilder, H., 379 (see Hoagland), *386*
Gillis, L., 381, *383*
Gilman, H., 28, *36*
Gjessing, E. C., 315
Gladding, J. K., 9 (see Milas), 15 (see Milas), 16 (see Milas), 18 (see Milas), 19 (see Milas), 22 (see Milas), 30 (see Milas), *37*, *38*

Glass, S. J., 419–422, *429*
Glavind, J., 168, *172*
Glazener, E. W., 315
Glick, D., 2, 27 (see Sobotka), *38*
Glock, G. E., 290, 295–297, *309*
Goettsch, M. A., 190, 191, *194*
Goff, B. L., 50 (see Clausen), 58 (see Clausen), *86*
Goldblatt, R., 307, *310*
Goldfarb, W., 64 (see Himwich), *87*
Goldman, A., 379, *385*
Goldman, J. L., 336, *385*
Goldsmith, E. D., 277, 279, 281–289, 294–299, 305, 307, *309–312*
Goldsmith, G. A., 126, 132, 136, 138, 140, 143–146, 148, 153, *158*, *159*
Golumbic, C., 59, *87*
Gomez, E. T., 260–262, *269*, *272*
Gonçalves, J. M., 292, *309*
Goodland, R. L., 65, *91*
Gorbman, A., 315
Gordan, G. S., 212, 222, *228*
Gordon, A. S., 277, 279, 281–287, 288 (see Keston), 289, 294–299, 305, 307 *309–311*
Gordon, E. F., 200, *229*
Gordon, M., 282 (see Goldsmith), 286 (see Goldsmith), *309*
Goss, H., 61, *87*
Gottfried, S. P., 50 (see Sobel), 58 (see Sobel), *91*
Gottlieb, H. F., 58, *87*
Goudsmit, J., 106, *118*
Gould, R. G., 22, *36*
Graham, R. M., 294, *313*
Grasso, R., 290, 292, *309*, 315
Grau, C. R., 79, *85*
Greaves, J. D., 58, 75, *87*
Green, R. G., 65, *87*
Greenblatt, R. B., 324, 329, 332, 337, 344–346, 351, 354, 357, 359, 362, *385*, *386*
Greene, D. J., 164 (2), *171*
Greene, R. R., 241, 242, 266, *269*, 321, 323, *386*
Greenhill, J. P., 329, 332, 343–346, 354, 356, *386*
Greep, R. O., 198, *229*, *230*
Griesbach, W. E., 276, 278–280, 282, 294, 295, 300, 306, *310*, 315
Griffith, W. H., 68, 78, 79, *87*
Griffiths, M., 247, *269*
Grijns, G., 93, *116*
Griswold, G. C., 397, 398 (see Klinefelter), 401 (see Klinefelter), *430*
Groody, M., 73 (see Morgan), *89*
Groome, J. R., 255, 256, *272*, 363, *386*
Gros, G., 238, 253, 255, *268*
Gross, F., 111, 113, *116*, *117*
Gross, J., 300, *311*
Grossi, F. X., 2 (see Milas), 4 (see Milas), 9 (see Milas), 15 (see Milas), 16 (see Milas), 18 (see Milas), 19 (see Milas), 22, 35, *37*, *38*
Grossman, W. I., 54, *88*
Grossowicz, N., 315
Grundy, W. E., 51 (see Denko), 72 (see Denko), 74 (see Denko), 75 (see Denko), 77 (see Denko), *86*
Guerrant, N. B., 66, 68, *87*
Guggenheim, K., 60, 68, *86*, *87*
Guirard, B. M., 46, *91*
Guiraud, P., 382, *386*
Gunderson, F. L., 82, *87*
Gusberg, S. B., 359, *386*
Gustavson, R. G., 243, 247, *271*, 276, 287 (see Wilgus), *314*
Guthkelch, A. N., 262, *269*, 365 (see Folley), *385*
Guttman, G. P., 225, *229*
Gwinner, G., 132 (see Spies), 154 (see Spies), *160*
György, P., 74, *87*, 183, *194*, 293, 307, *310*

H

Haag, J. R., 66 (see Weswig), *92*
Haas, A., 382, *386*
Haas, G. J., 69 (see Furter), 83 (see Furter), *86*
Hague, E., 46 (see du Vigneaud), *86*
Hair, L. Q., 324, *385*
Hale, H. B., 264, *272*
Hall, K., 267, *270*
Halpern, S. R., 248, *269*
Halverstadt, I. F., 303, *307*
Ham, T. H., 122 (see Castle), 126 (see Castle), 150 (see Castle), *157*
Hamblen, E. C., 327, 329, 383, *386*, 394 (see Pullen), *431*

Hamburger, C., 408, *429*
Hamilton, J. B., 251, 265, *269*, *272*, 328, 334, 337, 338 (see Edwards; Reynolds), *384*, *386*, *388*, 394, 398, 406, 408, 409, 418, 419, 425, 426, *428–430*
Hamilton, T. S., 50 (see Johnson; Shields; Spector), 78 (see Johnson), 79 (see Johnson), 84 (see Johnson), *88*, *91*, 156, *159*
Hamm, L., 423, *429*
Handelman, M. B., 200, *229*
Hanes, F. M., 124, *158*
Hanze, A. R., 16, 20, *36*
Harding, F. E., 413, 418, *429*
Hardy, S. M., 305, *314*
Harington, C. R., 290, *310*
Harley-Mason, J., 305, *308*
Harnly, M. H., 283, *309*
Harper, S. H., 35 (see Batty), *38*
Harrington, T. M., 35, *38*
Harris, P. L., 44, 59, *87*, *88*
Harris, P. N., 303, *308*
Harris, R. S., 5, 14, 27
Harrison, H. C., 212, *229*
Harrison, H. O., 358 (see Shorr), *389*
Harsh, R., 238, *269*
Hart, E. B., 40, 76 (see Moore), *87–89*, 155 (see Luckey), *159* (see Luckey), 163 (see Luckey), 164 (14–16, 26, 27, 43), 167 (see Hegsted), 168 (see Briggs; Hegsted; Luckey), 170 (see Briggs), 171 (see Briggs), *172*, *173*
Hart, P. C., 235 (see Laqueur), *270*
Hart, W. M., 292, 293, *312*
Hartman, C. G., 331, 333, 339, *384*, *386*
Hartzell, A., 284, *310*
Hartzler, E. R., 49, 63, *87*
Hasler, A. D., 65, *86*
Hastings, A. B., 141, 144, *160*
Haterius, H. D., 260, *269*
Hathaway, M. L., 49, 51, 52, 63, 67, *87*, *89*
Hauge, S. M., 285, 299, *313*
Hauschildt, J., 185, *194*
Hauschildt, J. D., 177, *194*
Hauser, G. F., 76 (see Daniel), *86*
Hawke, C. C., 398, 406 (see Hamilton), 408 (see Hamilton), 409 (see Hamilton), 429
Hawkins, E. G. E., 15, 34, *36*, *38*
Hawley, E. E., 49, 63, *87*
Haymaker, W., 374 (see Anderson), *383*
Hazel, G. R., 305 (see Van Winkle), *314*
Heckel, N. J., 240, *269*, 415, 416, 426, *429*
Heggie, R. S., 5, *37*
Hegsted, D. M., 164 (16, 26, 27), 167, 168, 169 (28), 171 (see Briggs), *172*
Heilbron, I. M., 1, 2, 4, 22 (see Davies), 27, 28, 35 (see Batty), *36*, *38*
Heilman, H., 226, *229*
Heiman, V., 164 (29, 46), 166, *172*, *173*
Heinle, R. W., 49 (see Welch), 77 (see Welch), *92*, 122 (see Suárez), 136, 138 (see Suárez), 140, 143 (see Moore), 144–148, 150, 151 (see Welch), 155 (see Welch), *158–161*
Heitman, H., Jr., 164 (35), *173*
Heller, C. G., 319, 326, 339, 358, *386*, 396–398, 401, 403, 407–415, 419, 424, *429*, *430*
Heller, E. J., 397, 407, 408, *429*
Hellman, A. M., 370, *386*
Hellwig, C. A., 300, *310*
Henderson, E., 393
Henderson, L. M., 67 (see McIntire), 70, *88*, *89*
Henry, G. W., 358, *386*, 420, *429*
Henry, K. M., 60 (see Booth), *85*
Hercus, C. E., 276, *310*
Hernandez-Beguerie, R. L., 134, *158*
Hernandez-Morales, F., 125 (see Spies), 132 (see Spies), *160*
Herring, V. V., 224, *229*
Herrmann, J. B., 333–335, 362, 372, *383*, *386*
Hertz, R., 156, *158*
Hertz, S., 275, 287, 288, *313*, 322, 378, 379 (see Kinsell), *386*
Heslin, J. E., 251, *269*
Heslop, R. N., 1, *36*
Hessel, F. H., 45 (see Scott), *91*
Heuser, G. F., 42 (see Scott), *91*, 121, *159*, 164 (3, 46, 56), 166, 167 (see Lucas), 168 (see Hill; Scott), 170 (see Scott), 171 (see Scott), *172*, *173*
Hickman, K. C. D., 44, 59, *87*
Higgins, G. M., 278–280, 284, 288, 295, 297, 307, *310*
Higgins, S. M., 221, *229*

Hill, F. W., 42 (see Scott), *91*, 168, *172*

Hill, R. T., 261, *271*, 419, *431*

Himes, H. W., 41 (see Melnick), 44 (see Melnick), 45 (156), 47 (see Melnick), 72 (see Melnick), 73 (see Melnick), *89*

Himwich, H. E., 64, *87*

Hines, D. C., 305 (see Van Winkle), *314*

Hines, L. R., 61, *87*

Hinton, J. W., 287 (see Lowenstein), *311*

Hirschberg, E., 72 (see Rubin), 73 (see Rubin), *90*, *91*

Hirschfeld, M., 419, *429*

Hirst, J. C., 348, *386*

Hisaw, F. L., 201 (see Fevold), *228*, 250, 251, *270*

Hoagland, C. L., 379, *386*

Hochberg, M., 41 (see Melnick), 43, 44 (see Melnick), 45 (156), 47 (see Melnick), 49, 52, 53, 54 (see Melnick; Oser), 62, 65 (see Melnick), 66, 72 (see Melnick), 73 (see Melnick), 80 (see Melnick), 84, *87*, *89*, *90*

Hodges, C., 426, *429*

Hodson, A. Z., 42, *87*

Hoff, H. E., 280, 285, 286, 295, 296, *311*

Hoffman, J., 275, 341, *386*

Hogan, A. G., 120, 121, 126, *158–160*, 164 (32), 169, 170 (see Richardson), *172*, *173*, 285, *312*

Hohlweg, W., 238, 239, 248, 250, *269*

Holder, D., 222, *229*

Holm, L. W., 297, 300, *309*, 315

Holmes, E. G., 128, *158*

Holmes, R. O., 225, *230*

Holt, L. E., Jr., 51, 52 (see Najjar), 67, 68 (see Najjar), 71 (see Najjar), 74 (see Najjar), 77 (see Najjar), *89*

Holter, H., 99, *117*

Hooker, C. W., 239, 240, *270*

Hopkins, F. G., 62, 63, *86*, *87*

Hopson, E. M., 279, *313*

Horning, E. S., 248, 249, 260, 262, 263, *268*

Horowitz, N. H., 45 (107), *87*

Horwitt, M. K., 40, *87*

Hoskins, E. R., 286, *310*

Hoskins, M. M., 286, *310*

Hoskins, R. G., 420, *431*

Hoskins, W. H., 328, *386*

Hotchkiss, R. S., 358 (see Shorr), *389*

Houssay, B. A., 213, *229*, 261, *270*, 274, *310*

Howard, J. E., 324, 377, *386*, *390*, *391*, 394, 412, 413, 419, *429*, *431*

Huber, W., 16 (see Isler), 20, 22, 35, *36*, *38*, 83, *87*

Hubert, G. R., 338 (see Edwards; Reynolds), *384*, *388*, 408, 409 (see Catchpole), 418, 419, *428–430*

Huckel, W., 33, *36*

Hueper, W. C., 286, 297, 301, *310*

de la Huerga, J., 69, 70 (see Krehl), 71 (see Krehl), 76 (see Krehl), *88*, 140 (see Krehl), *159*

Huff, J. W., 68 (see Sarett), 70 (see Rosen), 81 (see Rosen), 84, *90*, *91*

Huffman, J. W., 327, 329, 332, 343, 344, 346, *386*

Huffman, M. N., 234, *270*

Huggins, C., 240, *270*, 426, *429*

Huggins, R. A., 352, *386*

Hughes, A. M., 281–283, 285–287, 297, 303, 307, *308*, *310*

Huhner, M., 419, *429*

Hultquist, M. E., 119 (see Angier), 121 (see Angier), *157*

Humphreys, S., 70 (see Wintrobe), *92*

Hunter, G., 177 (see Jones), 182 (see Jones), *194*

Hunter, J. E., 164 (45), *173*

Hunter, R. F., 15, 34, *36*, *38*

Huret, G., 381, *387*

Hurst, A., 124, *158*

Hurxthal, L. M., 423, *429*

Hutchings, B. L., 76, *88*, 119 (see Angier), 120–122, 126 (see Day), *157–159*, 164 (33), 168, *172*

Hutt, F. B., 166, *173*

Hutton-Rudolph, M., 102, 114, *117*

I

Ichniowski, C. T., 286, 297, 301, *310*

Ingelfinger, F. J., 124, 132, *159*

Ingle, D. J., 221, 224, 225, *229*, 284, 288, 295, 297, *310*

Inui, F., 61 (see Poth), *90*

Isaacs, R., 133, 145–148, *159*

Isbell, E. R., 68, 69, *89*

Isbell, H., 74 (see Sydenstricker), *92*
Ishikawa, S., 2, *36*
Isler, O., 16, 20, 22, 35, *36*, *38*
Israëls, M. C. G., 143, 145 (see Wilkinson), 146, 148, *161*
Israel, S. L., 239, 244, *270*, 356, *386*
Ivy, A. C., 241, 242 (see Greene), 266 (see Greene), *269*

J

Jackson, A. S., 305, *310*
Jacobi, H. P., 79, *88*
Jacoby, A., 344, 345, 355, *386*
Jaffe, I., 418, *429*
Jailer, J. W., 285, *310*, *313*
James, L. H., 63, *92*
Jandorf, B. J., 280 (see Williams), 283, 285 (see Williams), 291, 295–299, 301, 302, 305 (see Williams), *310*, *314*
Janes, R. G., 296, 297, *310*
Jansen, B. C. P., 99, *117*
Jarboe, L., 352 (see Child; Woodbury), *384*, *391*
Jarboe, M., 352 (see Woodbury), *391*
Jeffreys, C. E. P., 63 (see Borsook), *85*
Jenesel, D. A., 303, *308*
Jenkins, G. N., 305, *308*
Jensen, J. L., 44 (see Harris), *87*
Jeppson, E. M., 370, *386*
Jewett, H. J., 324, *386*
Joël, C. A., 359, *386*
Joffe, M., 44, *87*, *88*
Johansson, D. R., 291, *312*
Johns, G. A., 51 (see Najjar), 52 (see Najjar), 68 (see Najjar), 71 (see Najjar), 74 (see Najjar), 77 (see Najjar), *89*
Johnson, A. W., 2, 4, *36*
Johnson, B. C., 78, 79, 84, *88*, *91*, 121, 156, *159*
Johnson, H. C., 122, 125, 127, 132, 135, 136, 149, *158*
Johnson, M. L., 49 (see Parsons), 50 (see Parsons), 66 (see Parsons), *90*
Johnson, R. E., 50 (see Sargent), *91*
Johnson, R. H., 164 (18), *172*, 419, 420, *429*
Johnson, T. B., 290, *310*
Johnson, W. W., 417, *429*
Johnston, C., 42 (see Deuel), *86*
Jolliffe, N., 58, 79, 80, *88*
Jones, E., 122, 123, 125, 127, 131–140, 143–146, 148, 149, 157, *158*, *159*
Jones, E. R. H., 2 (see Cymerman; Heilbron), 4 (see Heilbron), 28 (see Heilbron), *36*
Jones, G. E. S., 296, *310*
Jones, H. W., 341, *386*
Jones, J. H., 177, 182, *194*
Jones, W. E., 1, 2, 22 (see Davies), 27 (see Heilbron), 35 (see Batty), *36*, *38*
Joneson, O. R., 307, *310*
de Jongh, S. E., 235 (see Laqueur), 242, 247, 251, 255, 258, 260, 261, 264, 266, *268–270*, *272*
Joseph, M., 374 (see Anderson), *383*
Jucker, E., 13, 15, 28, *37*
Jürgens, R., 16
Juhn, M., 286, *310*, 315
Jukes, T. H., 72 (see Spies), 76, 79, *88*, *91*, 164 (34–36), 167, *172*, *173*, 183 (see Daft), *194*
Julius, H. W., 15 (see van Eckelen), *36*
Jull, M. A., 315
Jungck, E. C., 397, *429*
Jungherr, E., 165, *173*
Jurist, V., 45 (122), *88*

K

Kadow, P. C., 297, *310*
Kägi, H., 321 (see Miescher), *388*
Kaiser, P., 96, 111, *117*
Kalman, C., 222, *229*
Kaminester, S., 339 (see Reynolds), *389*
Kann, S., 42 (see Sobotka), *91*
Kantner, A. E., 370 (see Jeppson), *386*
Karrer, P., 1, 2, 13, 15, 27, 28, 30, *36*, *37*, 59, *88*
Kasabach, H. Y., 370 (see Jeppson), *386*
Kaser, M. M., 133, 137, 138, *158*
Katzen, P., 322, *384*, 412, *428*
Katzin, B., 224, *229*, 374 (see Long), *387*
Kaufmann, J., 143–146, 148, 149, *159*
Kavanagh, F., 46, *90*
Kay, G. A., 277, 278, 280 (see Williams), 283, 298, 301–303, *314*, 316
Kearns, W. M., 412, 426, *430*
Keating, F. R., Jr., 288, 294, *310*, *311*

Keene, F. E., 347, *386*
Kelly, E., 58, *89*
Kelly, E. C., 141, *159*
Kempster, H. L., 164 (32), 170 (see Richardson), *172*, *173*, 285, *310*
Kendall, E. C., 221, *229*, *230*
Kennaway, N. M., 236, 239, 254, *268*
Kennedy, T. H., 276–280, 282, 295, 296, 300, 306, *310*, *311*
Kenney, F. R., 305 (see Williams), *314*
Kensler, C. J., 67 (see Singher), *91*
Kenyon, A. T., 235 (see Gallagher), *269*, 328 (see Hoskins), 374, 377, *386*, 394, 396, 397 (see Gallagher), 413, 419, *428–430*
Keresztesy, J. C., 65, *88*
Kerrigan, S., 244, *272*
Kertesz, Z. I., 63, *88*
Keston, A. S., 288, 289, 291, *311*
Kety, S. S., 394, *428*
Keys, A., 50, *89*
Khayyal, M. A., 95 (see Gaddum), *116*
Kibrick, E. A., 200, 201 (see Evans), *228*, *229*
Kimbrough, R. A., 347, *386*
King, C. G., 43, 63 (see Schultze), *85*, *89*, *91*
Kingsley, G. V., 44 (see Ewing), *86*
Kinsell, L. W., 322, 335, 378, 379, *386*, *387*
Kinsey, A. C., 420, 422, *430*
Kipping, F. B., 2, *37*
Kirch, E. R., 50, *86*, *88*
v. Klaveren, F., 99 (see Kuhn), *117*
Kleiber, M., 212, *229*
Klein, J. R., 68 (see Sarett), 70, *91*
Kline, E. M., 49, 57, *86*
Kline, O. L., 52, 54, *88*
Klinefelter, H. F., Jr., 396–398, 401, 410, *430*
Klopp, C., 337, *387*
Klopp, C. T., 52, *88*
Klor, S. J., 370 (see Birnberg), *384*
Knandel, H. C., 164 (45), 170 (see Patrick), *173*
Knotts, F. L., 61 (see Poth), *90*
Knowlton, K., 374 (see Kenyon), 377 (see Kenyon), *386*, 419, *430*
Knox, W. E., 54, *88*
Koch, F. C., 234, 235 (see Gallagher), *269*, *270*, 328 (see Hoskins), 374 (see Kenyon), 377 (see Kenyon), *386*, 396 (see Gallagher; Kenyon), 397, 419 (see Kenyon), *429–431*
Koch, H. P., 32, *37*
Koch, M. B., 123, 125 (see Spies), 128 (see Spies), 132 (see Spies), 140, 142 (see Vilter), 143, 144, 145 (see Vilter), 146, 148, 155 (see Spies), *160*
Kochakian, C. D., 319, 321, 324, 325, 336–338, 374, 380, *387*, 414, *430*
Köster, H., 4, *37*
Kofler, M., 16 (see Isler), 22 (see Isler), 35 (see Isler), *36*, *38*
Kok, D. J., 255, *269*
Kon, S. K., 60 (see Booth), *85*, 370, *385*
Koneff, A. A., 249, *270*
Korenchevsky, V., 236, 239, 250, 252, 263, 264, 266, 267, *268*, *270*
Kornberg, A., 156, *159*, 296–298, 300, *308*, *309*, *311*
Koster, P., *36*
Kovitz, J., 2, *37*
Krafft-Ebing, R., 419, *430*
Kramer, B., 44 (see Sobel), 50 (see Sobel), 58 (see Sobel), *91*
Krampitz, L. O., 65, *88*, 189, *195*
Kratzer, F. H., 164 (6), 166 (see Bird), 170 (see Bird; Lepkovsky), *172*, *173*
Krauze, M. V., 2, *37*
Krehl, W. A., 47, 69–71, 76, 83, 84, *88*, 140, *159*
Kreula, M., 59, *88*
Kringstad, H., 58, *88*
Krohn, L., 351 (see Lackner), 353 (see Lackner), *387*
Krohn, P. L., 255, *272*
Kroom, D. B., 200 (see Freud), *228*
Kuder, A., 341, *390*
Kuether, C. A., 43, *90*
Kuh, E., 119 (see Angier), 121 (see Angier), *157*
Kuhn, H., 242, *272*
Kuhn, R., 1, 6, *37*, 99, *117*
Kun, H., 328, *390*
Kuizenga, M. H., 225 (see Ingle), *229*
Kulsavage, R. J., 307, *311*
Kummerow, F. A., 73 (see Quackenbush), *90*
Kurland, A. A., 416, 419, *431*

Kurzrok, L., 359, 360, 370 (see Birnberg), *384*, *387*

Kurzrok, R., 331, 351–353, 370, 374, *387*, *391*

Kuschner, J. I., 370, 374, *387*

L

Lacassagne, A., 239, 243, 253, 254, 258, 260, 262, *270*, 371, 372, *387*, 425, *430*

Lacey, R. N., 2 (see Cymerman), *36*

Lackner, J. E., 351, 353, *387*

Lafitte, A., 381, *387*

Lamar, V. K., 412, *430*

Lamont, A., 243, *271*

Lamoreux, W. F., 166, *173*

Landgrebe, F. W., 293, 300–302, *308*, *311*

Landwehr, G., 51, 67, *85*

Langston, W. C., 120, 126, 140 (see Day), 154 (see Day), *158*, *159*

Laqueur, E., 235, 242, 247, 260, 265, *268–270*, 395, 425, *428*

Laroche, G., 326, 328, 359, 360, *387*

Larson, R. A., 288, 294, *311*

Laskowski, S. C., 83 (see Huber), *87*

Lass, P. M., 370, *387*

Lattimer, J. K., 337, *387*

Lauter, W. M., 49 (see Furter), 52 (see Furter), 83 (see Furter), *86*

Lawson, F. L., 307, *311*

Lawson, H. A., 124 (see Castle), 132 (see Castle), 133 (see Castle), *157*

Leake, C. D., 191 (see Snell), *195*

Lease, E. J., 89, *89*

Lease, J. G., 89 (see Lease, E. J.), *89*

Leathem, J. H., 226, *229*, 258, 261, *271*, 280, 284, 285, 287, 295–297, *311*

Leblond, C. P., 280, 285, 286, 295, 296, 300, 307, *311*

Lee, C. O., 54, *92*

Lee, J. G., 169, *173*

Lee, J. T., 61 (see Poth), *90*

Lee, M., 201, *228*

Lee, M. O., 212, *229*

Lee, S. W., 2, 4, *37*

Leff, W. A., 344, 345, *387*

Legutke, G., 33 (see Huckel), *36*

Lehman, R. W., 44 (see Baxter), *85*

Leloir, L. F., 213, *229*

Lennon, B., 295 (see Rawson), *313*

Leonard, S. L., 222, *229*, 250, 251, 261, *270*

Lepkovsky, S., 64, *86*, 164 (6), 166 (see Bird), 170, *172*, *173*

Lerman, J., 288 (see Rawson), 292, 305 (see Rawson), *313*

Lerner, S. R., 289, 291, *309*, *311*

LeRosen, A. L., 32 (see Zechmeister), *38*, 42 (see Zechmeister), *92*

Lesser, M. A., 422, 423, *430*

Levie, L. H., 200, *228*, 246, *269*

Levin, L., 226, *229*, 285, 287, *312*

Levine, E. B., 424, *430*

Levine, S. A., 423, *430*

Levy, H., 67 (see Singher), *91*

Lewis, A. A., 262, *270*

Lewis, D., 365, *387*

Lewis, J. C., 207, *229*

Lewy, F. H., 114, *117*

Li, C. H., 198, 202, 204–209, 212, 213 (see Becks), 215, 216, 218, 220–222, 224 (see Ingle), 225, 226, *227–230*

Liang, T. Y., 226 (see Sayers), 227 (see Sayers), *230*

Lie, J., 58, *88*

Liechti, A., 96, *117*

Light, A. E., 46, *89*

Light, R. F., 52, *89*

Likoff, W. B., 423, *430*

Lillie, R. J., 286, *308*

Limarzi, L. R., 307, *311*

Lindberg, H. A., 275, *308*

Linderström-Lang, K. C., 99, *117*

Lindley, D. C., 74, *89*

Link, K. P., 61 (see Black), *85*

Lippincott, S. W., 181–185, *194*

Lippross, O., 426, *430*

Lipschütz, A., 248, 251, 265, *270*, 346, *387*

Lisser, H., 322–324, *384*, *385*, *387*, 412, *428*, *430*

Littlejohn, J. M., 70 (see Singal), *91*

Livingston, S., 359, *384*, *387*

Lobb, D. E., 52, 68, *87*

Loeser, A. A., 324, 326, 329, 333, 342, 343, 346, 353, 362, 367, 372, 378, *387*

Loewenstein, E., 42 (see Sobotka), *91*

Loewi, O., 94, 110, 114, *117*

Long, B., 46 (see du Vigneaud), *86*

Long, C. N. H., 198 (see Sayers; White), 199, 208, 212, 215, 218 (see Sayers), 220 (see Sayers), 224, 226 (see Sayers), 227 (see Sayers), *229–231*, 374, *387*
Long, J. A., 200, 201, *228*
Longsworth, L. G., 74, *92*, 204
Lopez, G. G., 125 (see Spies), 132 (see Spies), 154 (see Spies), 155 (see Spies), *160*
Lorenz, E., 324 (see Shimkin), *389*
Lotmar, W., 95 (see v. Muralt), *117*
Lotwin, G., 419 (see Kenyon), *430*
Lough, W. G., 287 (see Lowenstein), *311*
Lowe, A., 2 (see Davies), 22 (see Davies), 27 (see Heilbron), *36*
Lowe, C., 290, *314*
Lowenstein, B. E., 227, *229*, 287, *311*
Lucas, C. C., 78, *85*
Lucas, H. L., 167, *173*
Luckey, T. D., 76 (see Moore), *89*, 155, *159*, 163, 164 (14, 43), 168, 170 (see Briggs), 171 (see Briggs), *172*, *173*
Ludewig, S., 315
Lüthy, H., 99, 100, *117*
Lugg, J. W. H., 43, *89*
Lund, H., 30, *37*
Lurie, L. A., 420, *430*
Lyman, C. M., 115, *116*
Lynn, G. W., 282, 286, *311*
Lyons, C., 225, *231*
Lyons, W. R., 198, 216, 218, 220, *229*, 260–262, *270*, *271*

M

McAlevy, A. J., 2, *38*
McArthur, J. W., 295 (see Rawson), 306, *311*, *313*
McCall, K. B., 121 (see Cooperman), 122 (see Cooperman), *157*
McCarrison, R., 126, 154, *159*, 274, 275, 300, *312*
McCollum, E. V., 60, *89*, 276, *311*
McConnell, J., 316
McCoord, A. B., 50 (see Clausen), 58 (see Clausen), *86*
MacCorquodale, D. W., 234 (see Huffman), *270*
McCoy, E., 76 (see Moore), *89*
McCreary, J. F., 58, *89*
McCullagh, E. P., 322, 365, *387*, 412, 415, 417, *430*
McCutcheon, A. B., 417, *430*
McDonald, N. S., 2 (see Milas), 4 (see Milas), *37*
McElroy, L. W., 164 (36), *173*
McGavack, T. H., 285, 296–299, 305, *312*, *314*, 316, 424, *430*
McGinty, D. A., 261, *270*, 288, 289, 303, *308*, *312*, *313*
McGovran, E. R., 283, *312*
McGurl, F. J., 415, 417, *430*
McIlwain, H., 192, *194*
McIntire, J. M., 67, *89*
McIntosh, F. C., 109, *117*
McKay, E. A., 280, 288, 289, *313*
McKelvey, J. L., 122 (see Watson), *161*
McKennon, B. J., 419, *429*
Mackenzie, C. G., 276–282, 284, 285, 287, 293, 294, 298–301, 303, *311*, 315
Mackenzie, J. B., 276–282, 284, 285, 287, 293, 294, 298–301, 303, *311*
MacLachlan, E. A., 322, 377, *390*
McLellan, A. M., 251, *271*, 425, 426, *430*
McMahon, H. E., 225, *229*
McMillan, T. E., 419, *431*
McMillan, T. J., 43, *89*
McShan, W. H., 291, *312*
McVeigh, I., 45 (30), 47 (see Burkholder), *85*
Maddock, W. O., 319, 326, 339, *386*, 397, *429*
Magy, D. B., 212, *229*
Malcolm, J. L., 299, *311*
Malik, K. S., 60, *85*
Malkiel, S., 293, *311*
Mallory, M. E., 135, 155, *159*
Man, E. B., 285, *309*
Mandy, A. J., 344, 374, *387*
Mandy, T. E., 344, 374, *387*
Mann, C. W., 285, *311*
Mann, F. G., 296, 305, *308*
Mannering, G. J., 68, *89*
Manning, P. D. V., 120, *160*
Marañón, G., 377, *387*
Margiel, E., 359, *387*
Margolese, M. S., 323, 360, *387*
Marine, D., 275, 277, 287, 296–300, *308*, *311*, *312*

Mark, J., 412, *428*
Markee, J. E., 330, 339, 340, 342, 357, *387*
Markham, M. J., 379 (see Goldman), *385*, 425, *430*
Marks, H. P., 49, *85*
Marks, S., 32 (see Morrell), *38*
Marrian, G. F., 234, *270*
Martin, B. B., 46, *92*
Martin, G. J., 73, 75, 78, *89*, 185, *194*
Martin, S. J., 250, 251, 262, *270*
Marvel, J. P., 168 (see Patton), *173*
Marx, W., 199, 200, 201 (see Evans), 202, 208, 212, 221, 222 (see Becks), *227–230*
Mason, H. L., 111, *118*
Mason, H. S., 5, *37*
Mason, K. E., 44 (see Harris), *87*, 190, 191, *194*
Matsuura, T., 2, *36*
Matterson, L. D., 167 (see Scott), 169 (44), 170, *173*
Matthews, C. S., 247, *270*
Mattill, H. A., 59, 61, *87*
Mattis, P. A., 156 (see Wright), *161*
May, C. D., 58, *89*
May, L. G., 286, 297, *311*
Mayer, A. M., 44 (see Sobel), *91*
Mazer, C., 239, 244, *270*, 327, 329, 343, *387*
Mazer, M., 327, 329, 343, *387*
Meamber, D. L., 201, 202, *228*
Means, J. H., 274, 275, 287, 288, 305 (see Rawson), 306, *311–313*
Mecke, F. E., 328 (see Taylor), *390*
Mediary, G. C., 51 (see Najjar), 52 (see Najjar), 68 (see Najjar), 71 (see Najjar), 74 (see Najjar), 77 (see Najjar), *89*
Meek, W. J., 79 (see Jacobi), *88*
Meigs, J. V., 368 (see Nathanson), *388*
Meinke, W. W., 59, *86*
Melnick, D., 41, 42 (see Oser), 43 (see Hochberg; Oser), 44, 45 (156), 47, 49, 52–55, 60, 62 (see Hochberg), 64 (see Field; Robinson), 65, 66 (see Hochberg), 69 (see Oser). 72, 73, 80, 82–84, *86*, *87*, *89*, *90*
Mendel, L. B., 40, *87*
Menéndez, J. A., 125 (see Spies), 128 (see Spies), 132 (see Spies), 155 (see Spies), *160*
Menkin, V., 341, *387*
Meranze, D. R., 398, 405, 416, *428*
Merrill, P., 295 (see Rawson), *313*
Merryman, G. H., 425, *431*
Meserve, E. R., 32 (see Deuel), *36*
Metzger, N., 277, 287, *308*
Meyer, A. E., 284, 287, 297, 298, *312*
Meyer, F. L., 49, 63, *89*
Meyer, K., 207, *228*
Meyer, R., 347, *387*
Meyer, R. K., 250, 251, *270*, 291, *312*
Meyers, M. C., 49 (see Bethell), 77 (see Bethell), *85*, 151 (see Bethell), 155 (see Bethell), *157*
Mickelsen, O., 50, *89*
Miescher, K., 321, *388*, 412, *430*
Milanes, F., 125 (see Spies), 128 (see Spies), 132 (see Spies), *160*
Milas, N. A., 2, 4–6, 9, 15, 16, 18, 19, 22, 25, 27, 30, 35, *37*, *38*
Milhort, T. H., 64, 71, *89*
Miller, E. C., 73, *89*, 185–188, *194*
Miller, E. W., 247, *271*
Miller, J. R., 348, *388*
Miller, M. W., 164 (4), *172*
Miller, N. E., 419, *430*
Miller, R. A., 215 (see Bates), 220 (see Bates), *227*
Miller, W. H., 293, 303 (see Anderson), *307*, *312*
Mills, R. C., 164 (14–16, 26, 27, 67), 167 (see Hegsted), 168 (see Briggs; Hegsted), 171 (see Briggs), *172*, *173*, 183, *194*
Mimpriss, T. W., 417, *430*
Mims, V., 76 (see Totter), *92*, 120, 122, 126 (see Day), 135 (see Mallory), 140 (see Day), 150, 151, 154 (see Day), 155, *158*, *159*
Minnich, V., 125 (see Spies), 128 (see Spies), 132 (see Spies), 139 (see Moore), 140 (see Moore), 155 (see Spies), *159*, *160*
Minot, A. S., 152, *159*
Minot, G. R., 124, 139, 141, 142, 144, 147, *157*, *159*
Mintz, M., 331 (see Geist), *385*

Minz, B., 93–96, *116*, *117*
Mirsky, I. A., 215, *230*
Mitchell, H. H., 41, 50 (see Johnson; Shields, Spector), 69, 78 (see Johnson), 79 (see Johnson), 84 (see Johnson), *88*, *89*, *91*, 156, *159*
Mitchell, H. K., 68, 69, 76, *89*, 120, *159*
Mixner, J. P., 261, *271*, 280, 307, *312*, *313*
Mocquot, P., 351, 359, *388*
Moir, C., 352, *388*
Mollard, H., 380, *384*
Money, W. L., 315
Moniz de Bettencourt, J., 96, 111, *117*
Montpellier, J., 250, *271*
Moon, H. D., 215, 221, 224, *228*, *230*
Moore, C. H., 242, *268*
Moore, C. R., 242, 243, *271*, 412, *430*
Moore, C. V., 122, 123, 125, 133, 139–146, 148, 149, *159*
Moore, D. H., 285, 287, *312*
Moore, E. D., 288, *313*
Moore, P. R., 76, *89*, 155 (see Luckey), *159*, 163 (see Luckey), 164 (43), 168 (see Luckey), *173*
Moore, R. A., 251, *271*, 425, 426, *430*
Moore, T., 40, *89*
de Moraes, J., 288, *308*
Morf, R., 1, 30 (see Karrer), *37*
Morgan, A. F., 68, 73, 81, *89*
Morgan, C. L., 164 (50), 168, *173*
Morgan, E. J., 62, *87*
Morgan, T. N., 293, 300–302, *308*, *311*
Moricard, R., 346, 351, 359, *388*
Morrell, R. S., 32, *38*
Morris, C. J. O. R., 1, *37*
Morris, H. P., 175, 177, 180–186, *194*, 279, 282, 295, 296, 299, 306, *308*, *309*
Mortara, F., 362 (see Greenblatt), *386*
Morton, J. H., 305 (see McGavack), *312*
Morton, M. E., 284, 285, *312*
Morton, R. A., 1, *36*
Moseley, R. W., 286, 297, *311*
de Moss, W. R., 261, *272*
Mowat, J. H., 119 (see Angier), 121 (see Angier), *157*
Mu, J. W., 243, 261, *269*
Mühlbock, O., 241, *271*
Muelder, K. D., 58, *89*
Muellner, S. R., 337, *388*, 426, *430*
Muhrer, M. E., 285, *312*
Mulford, D. J., 79, *87*
Mulligan, R. M., 239, *271*
v. Muralt, A., 95–99, 103–106, 109–111, 114–116, *117*, *118*
v. Muralt, G., 106–108, *117*
Murlin, J. R., 374, *387*
Murphy, R. R., 164 (45), *173*
Musulin, R. R., 43, *89*
Myers, G. B., 358 (see Heller), *386*, 398, 408, 409, 412, 419, 424, *429*
Myerson, A., 419–421, *430*

N

Nachmansohn, D., 94, 105, 115, 116, *117*
Nadar, D. S., 329 (see Sturgis), 344 (see Sturgis), 345 (see Sturgis), 362 (see Sturgis), *390*
Najjar, V. A., 41, 51, 52, 67, 68, 71, 74, 77, *89*
Napier, L. E., 125, *159*
Nasset, E. S., 66, *91*
Nathanson, I. T., 326, 328, 332, 359, 366, 368, 372, *388*
Neal, A. L., 45, *90*
Nelson, A. A., 315
Nelson, D., 321 (see Greene), 323 (see Greene), 364, *386*
Nelson, E. M., 49 (see Welch), 66, 77 (see Welch), *92*, 122 (see Suárez), 136 (see Heinle), 138 (see Suárez), 147, 151 (see Welch), 155 (see Welch), *158*, *160*, *161*
Nelson, H. V., 49 (see Welch), 77 (see Welch), *92*, 151 (see Welch), 155 (see Welch), *161*
Nelson, W. O., 244, 250, 252, 259, 260, 262, 266, *267*, *271*, *388*, 396, 398, 401, 403, 407, 408, 410, 411, 413, 415, *429*, *430*
Ness, H. T., 49, 55, 66, *90*
Neustadt, R., 419–421, *430*
Newcomer, H. S., 305 (see Van Winkle), *314*
Nicholson, J. T. L., 49 (see Reinhold), 64 (see Reinhold), *90*
Nielsen, E., 74, 76, *90*, 188, *194*, 212, *227*
Nigrelli, R. F., 282, 286, *309*, *312*
Nixon, W. L., 297, *314*
Noble, G. K., 281, *312*

Noble, R. L., 221, *230*, 245, *271*
Noonan, W. J., 419, *428*
Norberg, B., 95, *117*
Norris, E. R., 185, *194*
Norris, L. C., 42, 76 (see Daniel), *86*, *87*, *91*, 121, *159*, 164 (3, 46, 55, 56, see Cravens), 166 (see Heuser), 167 (see Lucas), 168 (see Hill; Scott), 169 (see Cravens), 170 (see Scott), 171 (see Scott), *172*, *173*
Northey, E. H., 119 (see Angier), 121 (see Angier), *157*
Norton, S. G., 324 (see Shimkin), *389*
Novak, E., 339, 346, 347, 351, *388*
Nowinski, W. W., 292, *309*
Nowotarski, J. S., 166, *173*

O

Oberndorfer, S., 398, *430*
O'Connell, C. P., 370, 374, *387*
O'Dell, B. L., 120 (see Pfiffner), 121 (see Pfiffner), 126, 151 (see Pfiffner), *159*, *160*, 164 (32), *172*
Österreicher, W., 421, *430*
Oestreicher, F., 216 (see Reiss), *230*
Ogden, F. N., 123, 153, *161*
Ogg, C. L., 83 (see Badgett), *85*
Okkels, H., 339, *388*, 398, 420, 421, *431*
Olcott, H. S., 207, *229*
Oldham, H. G., 68 (see Davis), *86*
Oleson, J. J., 46, 76 (see Hutchings), *88*, *90*, 164 (33), 165, 168 (see Hutchings), *172*
Olsson, N., 166, *173*
Oppel, T. W., 51, 74, *90*
Oppenheimer, E., 321 (see Greene), 323 (see Greene), *386*
Ornstein, E. A., 413, *428*
Oroshnik, W., 22, *38*
Orr, J. W., 247, *271*
Orsini, D., 68 (see Mannering), *89*
Oser, B. L., 41–43, 44 (see Melnick), 45 (156), 47 (see Melnick), 49, 52–55, 57, 60, 62 (see Hochberg), 65 (see Melnick), 66 (see Hochberg), 69, 72 (see Melnick), 73 (see Melnick), 80 (see Melnick), 84 (see Melnick), *87*, *89*, *90*
Oser, M., 57, *90*
O'Sullivan, D. G., 28 (see Heilbron), *36*
Overholser, M. D., 238, 252, 266, *269*, *271*
Overman, R. S., 61 (see Black), *85*
Owen, S. E., 425, *430*

P

Pace, J. M., 418, *431*
Pader, M., 41 (see Oser), 42 (see Oser), 43 (see Oser), 69 (see Oser), *90*
Paes, E., 96, *117*
Palacios, L., 248, *270*
Palmer, H. D., 334, 346, *388*
Palmer, R., *388*
Papanicolaou, G. N., 326 (see Shorr), 328, 330–333, 335, 337, 359, 362, *388*, *390*
Pappenheimer, A. M., 191, *195*
Park, E. A., 60 (see McCollum), *89*
Parkes, A. S., 222, *230*, 233, 238, 244–246, 252, 253, 255, 257, 265, *268*, *269*, *271*, *272*, 324, *384*, 413, 425, *428*, *432*
Parrott, E. M., 120, *158*
Parsons, H. T., 49, 50, 51 (see Gardner), 52, 55 (see Ness), 64, 66, 69, 72, 74, *86*, *90*, *92*
Parsons, L., 338, 368 (see Nathanson), 375, *388*
Parsons, W., *383*
Paschkis, K. E., 278, 285, 291–293, 297, 298, 300–302, 305, *312*
Patrick, H., 164 (32, 50), 168, 170, *172*, *173*
Patton, A. R., 168, *173*, 276, 287 (see Wilgus), *314*
Patton, E. W., 139, *161*
Pauling, L., 32, *38*, 42 (see Zechmeister), *92*
Pavcek, P. L., 74, *90*
Payne, G. C., 124 (see Castle), 132 (see Castle), 133 (see Castle), *157*
Payne, R. L., 315
Peacock, W., 275, 288, 294, *310*, *311*, *313*
Peacock, W. C., 288 (see Rawson), 305 (see Rawson), *313*
Pearsons, J., 275, 287, *313*
Pencharz, R. I., 260, 262, *270*
Pepkowitz, L. P., 43, *90*
Peralta Ramos, A., 370, *388*

Perloff, W. H., 346, 375, *388*

Perlzweig, W. A., 68 (see Sarett), 69, 70, 81 (see Rosen), 84, *90*, *91*

Petering, H. G., 168 (see Patton), *173*

Peters, J. B., 280, 284, 285 (see Williams), 295–298, 305, *314*

Peters, R. A., 115, *117*

Petersen, D. H., 235 (see Gallagher), *269*

Petersen, W. E., 262, *271*

Peterson, D. G., 396, 397 (see Gallagher), *429*, *430*

Peterson, J. C., 153, *159*, *160*

Peterson, W. H., 49 (see Parsons), 50 (see Gardner; Parsons), 51 (see Gardner), 69 (see Parsons), 72 (see Parsons), 74 (see Gardner; Nielsen; Parsons), *86*, *90*, 120, *160*

Pfaltz, H., 16

Pfeiffer, C. A., 236, 239, 240, 247, 264, *269*, *270*

Pfiffner, J. J., 45 (23), 47 (see Bird), *86*, 120, 121, 151, *157*, *160*, 260, *271*

Phelps, D., 248, *272*

Phillips, E. O., 32 (see Morell), *38*

Phillips, F. S., 301, 307, *309*

Phillips, P. H., 114, *116*, 183 (see Mills), *194*

Pierre-Bourgeois, 380, *388*

Pillsbury, D. M., 381, *389*

Pinto, A., 239, *269*

Pipes, G. W., 302, *312*

Piquett, P. G., 283, *312*

Pirani, C. L., 307, *311*

Pitt, R. B., 9 (see Milas), 15 (see Milas), 16 (see Milas), 18 (see Milas), 19 (see Milas), 22, *38*

Plati, J. T., 4 (see Milas), 9 (see Milas), 15, 16 (see Milas), 18 (see Milas), 19 (see Milas), 22 (see Milas), 25, *37*, *38*

Platz, B. R., 73 (see Quackenbush; Schneider), *90*, *91*

Plummer, H. S., 274, *312*

Polgár, A., 42, *86*, *90*, *92*

Poling, C. E., 183, *194*

Pollano, O., 365, *388*

Pollock, J., 381 (see Feldman), *385*

Polskin, L. J., 58 (see Russell), *91*

Pomerene, E., 190, *194*

Poore, E., 67 (see Singher), *91*

Porter, J. W., 51 (see Denko), 72 (see Denko), 74 (see Denko), 75 (see Denko), 77 (see Denko), *86*

Portes, L., 370, *388*

Postle, B., 381 (see Rothermich), *389*

Poth, E. J., 61, *90*

Prados, M., 114, *118*

Prager, J. B., 381, *388*

Prato, G. S., 275, 287, *313*

Pratt, J. P., 371, *390*

Preissecker, E., 370, *388*

Prescott, F., 111, 114 (see Chamberlain), *116*

Pressman, D., 33 (see Winstein), *38*

Price, D., 243, *271*

Price, E. L., 49 (see Ness), 55 (see Ness), 66 (see Ness), *90*

Prickett, C. D., 178, *195*

Prietzel, F., 381, *388*

Pritchett, C. P., 422, *428*

Provost, R. C., Jr., 83 (see Badgett), *85*

Prudente, A., 372, *388*

Pullen, R. L., 394, *431*

Purves, H. D., 276–280, 282, 294–296, 300, 306, *310–312*, 315

Pybus, F. C., 247, *271*

Q

Quackenbush, F. W., 58 (see Gottlieb), 73, *87*, *90*

Quaife, M. L., 44, *90*

R

Raab, W. J., 296, 307, *313*

Rabbiner, B., 344, 345, 355, *386*

Radman, H. M., 359 (see Silberman), 362 (see Silberman), *390*

Rakoff, A. E., 278, 291–293, 297, 298, 300–302, *312*

Ramos, A., *388*

Randall, F. E., 45 (see Scott), *91*

Rannefeld, A. N., 47, 72, *91*

Ransom, G. V., 284, 287, *312*

Rao, M. N., 169 (28), *172*

Rardin, T. E., 422, *428*

Rasmussen, A. F., Jr., 154 (see Waisman), *161*

Raush, E. C., 261, *267*

Rawlings, L. B., 418, *431*
Rawson, R. W., 275, 287, 288, 294, 295, 305, 306, *310*, *311*, *313*, 316
Ray, R. D., 200, 213 (see Becks), *227*, *230*
Raynaud, A., 241, 258, 262, *270*, *271*, 372, *387*
Rea, C. E., 418, 419, 426, *428*, *431*
Rea, J. L., 1 (see Heilbron), *36*
Record, P. R., 164 (5, 52, 53), 165, 167, *172*, *173*
Reder, R., 62, 63, *90*
Reece, R. P., 261, *271*
Reed, W. A., 419, *431*
Regan, J. F. W., 244, *267*
Regen, E. N., 213, *231*
Reichstein, T., 222, *230*
Reifenstein, E. C., Jr., 225, *227*, 322, 335 (see Kinsell), 337, 377, 378, 379 (see Kinsell), *383*, *386–388*, 396, 398, 401 (see Fraser), 407 (see Fraser), 408 (see Fraser), 410, 411, *429–431*
Reinecke, R. M., 262, *271*
Reineke, E. P., 280, 291, 307, *312*, *313*
Reinert, M., 96 (see Liechti), *117*
Reinhardt, W. O., 216 (see Simpson), 225, 226, *229*, *230*, 284 (see Morton), *312*
Reinhold, J. G., 49, 64, *90*
Reiss, M., 216, *230*
Rennie, T. A. C., 419, *431*
Řeřábek, J., 298, *313*
Rettger, L. F., 63, *92*
Reveno, W. S., 278, 280, *313*
Reyniers, J. A., 190, *195*
Reynolds, S. R. M., 338, 339, 351, *388*, *389*
Rhoads, C. P., 52 (see Klopp), 67 (see Singher), *88*, *91*, 124 (see Castle), 132 (see Castle), 133 (see Castle), *157*
Richardson, A. M., 376 (see Albright), *383*
Richardson, L. R., 164 (32), 170, *172*, *173*
Riches, S. W., 426, *431*
Richter, C. P., 276–278, 298–301, 307, *309*, *313*, 315
Riddell, C. B., 288 (see Rawson), 294, 295 (see Rawson), *313*
Riddle, M. C., 132, 145, 147 (see Isaacs), 148 (see Isaacs), *159*, *160*
Riddle, O., 215, 220, *227*
Ridout, J. H., 78, *85*
Riley, I. D., 381, *389*
Ringrose, A. T., 164 (46), *173*
Ringrose, R. C., 164 (55), *173*
Ripley, H. S., 331, 333, *388*
Riseman, J. E. F., 423, *431*
Ritsert, K., 106, *117*
Rivers, J. T., 9 (see Milas), 15 (see Milas), 16 (see Milas), 18 (see Milas), 19 (see Milas), 22 (see Milas), *38*
Rivers, J. T., Jr., 6, 22, *38*
Robbins, M., 45 (23), 47 (see Bird), *85*
Robbins, W. J., 46, *90*
Roberts, L. J., 68 (see Davis), *86*
Robertson, E. I., 164 (18, 56), *172*, *173*
Robertson, W. v. B., 180, 181, *194*
Robeson, C. D., 32, 33, 35, *38*, 44 (see Baxter), *85*
Robinson, P., 50 (see Sargent), *91*
Robinson, W. B., 43, *90*
Robinson, W. D., 49, 63, 65, 82 (see Melnick), 84 (see Melnick), *86*, *89*, *90*
Roblin, R. O., Jr., 293, 303 (see Anderson), *307*, *312*
Robson, J. M., 240, *268*, 330, 332, 370, *389*
Roe, J., 43, *90*
Rogers, P. V., 315
Ronco, A., 16 (see Isler), 20 (see Isler), 22 (see Isler), 35 (see Isler), *36*, *38*
Rose, C. S., 74, *87*
Rose, E., 275, *309*, 316, 375 (see Perloff), 381, *388*, *389*
Rose, W. C., 191, *195*
Rosen, F., 70, 81, *90*
Rosenberg, H. R., 190, *195*
Rosenburg, A., 365, *389*
Rosenzweig, S., 420, *431*
Ross, J. B., 122 (see Castle), 126 (see Castle), 132, 150 (see Castle), *157*
Rossine, J. A., 94, *117*
Rossmiller, H. L., 365, *387*, 415, *430*
Roth, A. A., 396, 398, 407, *429*
Rothbart, H., 360, *387*
Rothen, A., 198, *130*
Rothenberg, M. S., 278, 300, *312*
Rothermich, N. O., 326, 332, 381, *389*
de Rover, H., *36*

Rowntree, J. I., 57, *90*
Rubenstein, B. B., 361, *383*
Rubenstein, H. S., 344, 355, 362, *389*
Rubin, 331
Rubin, L., 287 (see Wolff), 288 (see Wolff), 289 (see Wolff), *314*
Rubin, M., 168, 171, *172*, *173*
Rubin, S. H., 49 (see Furter), 52 (see Furter), 54, 69 (see Furter), 72, 73, 83 (see Furter), *86*, *90*, *91*
Rubinstein, H. S., 415, 416, 418, 419, *431*
Rüegger, A., 2, 27, *37*
Ruegamer, W. R., 121 (see Cooperman), 122 (see Cooperman), *157*
Ruehle, A. E. J., 99, *117*
Ruffin, J. M., 133, *157*
Ruinen, F. H., 261, *271*
Rusch, H. P., 264, *271*
Russell, J. A., 213, *230*
Russell, R. A., 66, *91*
Russell, W. C., 58, *91*
Rutherford, R. N., 371, *389*
Rutschmann, J., 13, *37*
Ruz, O., 346 (see Lipschütz), *387*
Rydin, H., 95 (see Gaddum), *116*

S

Saethre, H., 408, *431*
St. John, J. L., 164 (18), *172*
Sakal, E., 2, 4 (see Milas), 9, 15, 16, 18, 19, 22, 25, 27, 30 (see Milas), *37*, *38*
Salaber, J. A., 344, 348, *384*, *389*
Salmon, A. A., 327 (see Salmon, U. J.), *389*
Salmon, U. J., 322, 326, 327, 329, 331 (see Geist), 332, 333, 335, 336, 343, 345, 346, 348, 351, 354, 357, 359, 360, 362, *385*, *389*, 408, *428*
Salmon, W. D., 73, *91*, 183, *195*
Salomon, H., 30 (see Karrer), *37*
Salter, W. T., 274, 280, 288, 289, 292, *313*
Sampson, J. A., 347, *389*
Sampson, W. L., 65, *88*
Samuels, L. T., 262, *271*
Sand, K., 398, 420, 421, *431*
Sandiford, I., 374 (see Kenyon), 377 (see Kenyon), *386*, 419 (see Kenyon), *430*
Sandoval, A., 32 (see Deuel), *36*
Sandza, J. G., 185, 192, *195*
Sanz, M., 106, *117*
Sardi, J. L., 348 (see Salaber), *389*
Sarett, H. P., 45 (226), 68, 70, 84, *91*
Sargent, F., 50, *91*
Sarkander, H., 96, *116*
Sarma, P. S., 47, 69, 73, 84 (see Krehl), *88*, *91*, 167, *173*
Saslaw, S., 121, *160*
Satterfield, G. H., 52 (see Kline), *88*
Sayers, G., 198, 215, 218, 220, 226, 227, *230*
Sayers, M. A., 226, 227, *230*
Schaaf, E., 28 (see Ziegler), *38*
Schacher, J., 225, *230*
Schachner, H., 289, 291, 303, *313*
Schaefer, W. H., 281, *313*
Schaffer, A. I., 379 (see Goldman), *385*
Schaffer, N. K., 212, *229*
Scharf, G., 261, *271*
Scheiner, J., 72 (see Rubin), 73 (see Rubin), *90*, *91*
Schenker, V., 414, *428*
Schick, E., 28, *37*
Schlessinger, H. I., 35, *38*
Schloss, S., 339 (see Reynolds), *389*
Schmidt, C. L. A., 58, 75, *87*, *91*
Schmidt, W., 58, *91*
Schmitt, F. O., 100, *117*
Schneider, H., 73, *91*
Schnetzler, E. E., 283, 285, 286, 297, 299, 302, *307*, *313*
Schoeller, W., 238, 244, *269*, *271*
Schöpp, K., 1, *37*
Scholz, C., 321 (see Miescher), *388*
Schreiber, E., 277, *314*
Schrire, I., 376, *389*
Schröder, R., 339, *389*
Schroeder, W. A., 42 (see Zechmeister), *92*
Schuerch, C., Jr., 4 (see Milas), *37*
v. Schulthess, G., 109, *117*
Schultz, A. S., 45 (10, 11, 235), 46, 52 (see Light), 72 (see Atkin), *85*, *89*, *91*, 99, *116*
Schultze, A. B., 260, *272*, 277, 283, 285, 286, 296, 297, 299, 304, 307, *313*
Schultze, M. O., 63, *91*
Schumann, W., 28 (see Ziegler), *38*

Schutzer, S., 316
Schwab, J. L., 121 (see Saslaw), *160*
Schwabe, E. L., 247, *270*
Schwager, P. G., 143–146, 148, 149, *159*
Schwarz, J., 265 (see Lipschütz), *270*
Schweigert, B. S., 67 (see McIntire), *89*
Schwenk, E., 317
Schwimmer, D., 285 (see McGavack), 305 (see McGavack), *312*
Scott, H. M., 167, 169 (44), 170 (see Matterson), *173*
Scott, M. B., 43, 44, *85*
Scott, M. L., 42, 45, 76 (see Daniel), *86*, *91*, 168, 170, 171, *172*, *173*
Scott, W. W., 396, 408, 409, *431*
Scow, R. O., 208, *230*
Sealock, R. R., 64, 65, 81, *85*, *91*
Sebrell, W. H., 60 (see Daft), 74 (see Daft), 76, *86*, 121, 122 (see Watson), 135, 156, *157–159*, *161*, 183 (see Daft), *194*, 297, 298, 300, *307*, *308*, *311*
Seckinger, D. L., 331, *389*
Seeger, D. R., 119 (see Angier), 121 (see Angier), *157*
Segaloff, A., 266, *271*, 299, *313*
Sellers, A. L., 424, *430*
Selye, H., 202, 225, *228*, *230*, 237, 238, 246, 260, *267*, *268*, *271*, 327, *389*
Semb, J., 119 (see Angier), 121 (see Angier), *157*
Severinghaus, A. E., 248, 249, *271*, *389*
Sevringhaus, E. L., 321, 326, *389*, 397, 407, 408, 419, 421, *429*, *431*
Shank, R. E., 379 (see Hoagland), *386*
Shantz, E. M., 6, 15, 17, 35 (see Cawley), *36*, *38*
Shapiro, H. D., 362 (see Rubenstein), *389*
Sharp, E. A., 122, 150, *160*, 289, 305 (see Van Winkle), *312*, *314*
Sharp, P. F., 43, 44, *91*
Sharpey-Schafer, E. P., 251, *271*, 359, 376, *389*
Sharpless, G. R., 275, 279, 287, *313*
Shaw, J. H., 183 (see Mills), *194*
Shedlovsky, T., 198, *230*
Shelton, E. K., 412, *431*
Sheppard, R., 225, *229*
Sherman, W. C., 59, *91*
Sherwood, R. M., 164 (61–63), 166 (see Couch), *172*, *173*
Shields, J. B., 50, *91*
Shimkin, M. P., 324, *389*
Shipley, P. G., 60 (see McCollum), *89*
Shipley, R. A., 207, 215, *230*
Shohl, A. T., 60, *91*
Shorr, E., 233, 285, *308*, *313*, 317, 323, 326–328, 330–333, 335, 337, 342, 357–359, 362, 375, 376, *384*, *386*, *388–390*
Shourie, K. L., 69, *91*
Shukers, C. F., 120 (see Langston), 126 (see Langston), 144, 150, 154, *159*, *160*
Shull, G. M., 74, *90*
Shumacker, H. B., 243, *271*
Shute, E., 373, *390*
Sickels, J. P., 119 (see Angier), 121 (see Angier), *157*
Siegler, S. L., 370, *390*
Sigler, L. H., 423, *431*
Sikkema, S., 321, *389*
Silber, R. H., 50, 72, *91*, *92*
Silberman, D., 359, 362, *390*
Silberman, S., 265 (see Lipschütz), *270*
Silverstein, L. M., 370, *390*
Sim, W. S., 32 (see Morrell), *38*
Simmonds, N., 60 (see McCollum), *89*
Simon, A. F., 327, 344, *390*
Simond, A., 243, *268*
Simonnet, H., 326 (see Laroche), 328 (see Laroche), 359 (see Laroche), 360 (see Laroche), *387*
Simpson, M. E., 198–202, 204 (see Li), 205 (see Li), 206 (see Li), 207 (see Evans; Li), 208, 209, 212, 213, 215, 216, 218, 220–222, 225, *227–230*, 249 (see Koneff), *270*
Simpson, S. L., 236 (see Korenchevsky), 267, *270*
Singal, S. A., 70, 74 (see Sydenstricker), *91*, *92*
Singer, M., 282, 292, *309*
Singher, H. O., 67, *91*
Singsen, E. P., 167 (see Scott), 169 (44), 170 (see Matterson), *173*
Sisk, W. N., 305 (see Van Winkle), *314*
Skeggs, H. R., 45 (248, 291), 47 (see Wright), *91*, *92*, 156 (see Wright), *161*
Skei, T., 33 (see Young), *38*

Slaughter, G., 426, *428*
Sloan, M. H., 285, *313*
Sloane, N. H., 120 (see Hutchings), 122 (see Day), 126 (see Day), *158*, *159*
Slobodin, Y. M., 2, *37*
Slobodkin, N. H., 120 (see Hutchings), 121 (see Hutchings), 122 (see Hutchings), *159*
Smelser, G., 285 (see Jailer), *310*
Smelser, G. K., 260, *271*, 285, 287, *312*
Smirk, F. H., 296, *309*
Smith, B. F., 111, *118*
Smith, G. V., 234 (see Huffman), *270*
Smith, G. Van S., 341, *390*
Smith, J. M., Jr., 119 (see Angier), 121 (see Angier), *157*
Smith, M. C., 43, *91*
Smith, O. W., 234 (see Huffman), 249, 250, *270*, *271*, 341, *390*
Smith, P. E., 200, 215, *230*, 415, *431*
Smith, P. H., 376 (see Albright), *383*, 397, *431*
Smith, R. E., 417, *431*
Smithcors, J. F., 296, *313*
Snell, E. E., 46, 47, 72, 73 (see Sarma), 74 (see György), 76 (see Mitchell), *87*, *89*, *91*, 120, *159*, *160*, 191, *195*
Sobel, A. E., 43, 44, 50, 58, *91*
Sobotka, H., 1, 2, 27, *38*, 42, 50, 58, *85*, *91*, 135, *157*
Soifer, S., 425, *431*
Solomon, S., 370, *383*
Somogyi, J. C., 62, 63, *91*
Soodak, M., 193, *195*
Soskin, S., 351 (see Lackner), 353 (see Lackner), *387*
Sotier, A. L., 45 (see Bauernfeind), *85*
Southam, A. H., 418, *431*
Spath, A., 28, *38*
Spector, H., 50, 69, *91*
Speert, H., 364, 365, *390*
Spence, A. W., 275 (see Marine), *311*, 368, *390*, 412, 419, *431*
Spencer, J., 243, 247, *271*
Sperry, W. M., 285, *307*, *310*, *313*
Spies, T. D., 72, *91*, 121–123, 125, 128, 132, 134, 136, 138, 139 (see Moore), 140, 141, 142 (see Vilter), 143–146, 148, 150, 154, 155, *157–161*
Spilzinger, J. M., 381, *390*
Spinks, A., 2 (see Heilbron), 4 (see Heilbron), *36*
Spiridanoff, S., 191 (see Snell), *195*
Sprague, K. L., 156 (see Wright), *161*
Sprince, H., 191, *195*
Stämpfli, R., 99, *118*
Stamler, F. W., 167, *173*
Stanbery, S. R., 72 (see Spies), *91*
Stander, H. J., 317
Stanley, M. M., 304
Starkey, W. F., 258, *271*
Steenbock, H., 58 (see Gottlieb), 59 (see Lease), 73 (see Quackenbush; Schneider), *87*, *89–91*
Stein, H. J., 70 (see Wintrobe), *92*
Steinach, E., 242, 259, *272*, 328, *390*
Steinbach, H. B., 105, 116, *117*
Steinitz, E., 418, *432*
Steinmetz, C. R., 240, *269*
Stekel, W., 420, *431*
Stephens, D. J., 49 (see Hawley), 63 (see Hawley), *87*
Sterne, G. D., 294, *313*
Stewart, A. P., Jr., 43, 44, *91*
Stewart, F. W., 365, 366, 371, *385*
Stewart, H. L., Jr., 371, *390*
Stiller, E. T., 293 (see György), *310*
Stimmel, B. F., 326 (see Shorr), 328, 330, 332, 335, 359, 362, *390*
Stirn, F. E., 64, *91*
Stokes, J. L., 45, 46, *92*
Stokstad, E. L. R., 61, 76 (see Hutchings), *85*, *88*, 119 (see Angier), 120, 121 (see Angier; Hutchings), 122 (see Day; Hutchings), 126 (see Day), *157–160*, 164 (33), 168 (see Hutchings), *172*
Stone, E. T. R., 349, *390*
Stone, R. E., 138, *160*
Stora, R., 382, *386*
Stotz, E., 43, 63 (see Schultze), *90*, *91*
Strauss, W., 59, *88*
Strauss, 111, *117*
Strauss, M. B., 141, 142, 147, *159*
Strohm, J. G., 425, *431*
Strom, J. E., 52, 67, *87*
Stromme, W. B., 371, *390*
Strong, F. M., 45, 47, 49 (see Parsons), 50 (see Parsons), 69 (see Parsons), 74 (see Parsons), 83, *88*, *90*, *92*

Strong, G. F., 423, *431*
Sturgis, S. H., 329, 344, 345, 351, 353, 362, *390*
Sturgis, W. A., *390*
Suárez, R. M., 122, 125 (see Spies), 132, 136, 138, *160*
Suárez, R. M., Jr., 122 (see Suárez), 125 (see Spies), 132, 136, 138 (see Suárez), *160*
SubbaRow, Y., 119 (see Angier), 120 (see Hutchings), 121 (see Angier), 141, 144, *157*, *159*, *160*
Sulkowitch, H., 396 (see Fraser), 398 (see Fraser), 401 (see Fraser), 407 (see Fraser), 408 (see Fraser), *429*
Sullivan, J., 277–279, 287, 294, 303, *308*
Sullivan, M. X., 44, *86*
Sulman, F., 412, *432*
Sumner, E., 42 (see Deuel), *86*
Sunderman, F. W., 375 (see Perloff), *388*
Sure, B., 67, *92*, 181, *195*
Sutton, L. A., 347, *390*
Swaminathan, M., 69, *91*
Swank, R. L., 114, *118*, 169, *173*, 176, *195*
Swendseid, M. E., 49 (see Bethell), 77 (see Bethell), *85*, 151 (see Bethell), 155 (see Bethell), *157*
Swern, D. E., 15, *38*
Sydenstricker, V. P., 70 (see Singal), 74, *91*, *92*

T

Taber, E., 297, *313*
Tager, B. N., 412, *431*
Tailhefer, A., 367, *390*
Talbot, N. B., 322, 377, *390*, 396, *431*
Tannheimer, J. F., 275, 288, *313*
Tappe, W., 33 (see Huckel), *36*
Taurog, A., 287 (see Wolff), 288 (see Wolff), 289 (see Wolff), 290, 293, 303, *314*
Tausk, M., 247, *272*
Taylor, H. C., Jr., 328, 337, 365–368, *387*, *390*
Taylor, H. W., 58 (see Russell), *91*
Taylor, J. D., 44 (see Baxter), *85*
Teel, H. M., 201, 211, *230*
Tennet, D. M., 50, *92*
Teply, L. J., 69 (see Krehl), 84 (see Krehl), *88*
Tepper, A. E., 164 (66), 165, *173*
Terry, D. E., 45 (see Andrews), *85*
Terry, G. S., 286, *314*
Tewkesbury, L. B., 290, *310*
Thayer, S. A., 234 (see Huffman), *270*, 397, *431*
Thomas, H. B., 419, *431*
Thomas, O. L., 292, *314*
Thompson, A. F., Jr., 22, *36*
Thompson, H. O., 54, *92*
Thompson, P. K., 305, *314*
Thompson, W. O., 305, *314*
Thomson, D. L., 202 (see Collip), 215 (see Collip), *228*, 246, 261, *268*, *271*
Thorn, G. W., 377, *390*
Tidrick, R. T., 167 (see Stamler), *173*
Tighe, L. W., 164 (29), 166, *172*
Tillson, E. K., 291, 297, 298, 301, 302, 305, *312*
Tipton, S. R., 297, *314*
Titus, H. W., 164 (see Cravens), 169 (see Cravens), *172*
Toca, R. L., 132 (see Spies), 154 (see Spies), *160*
Todhunter, E. H., 43, *89*
Todhunter, E. N., 49, 63, *92*
Torbet, N., 71, 76, *88*, 140 (see Krehl), *159*
Torpin, R., 352 (see Child; Woodbury), 362 (see Greenblatt), *384*, *386*, *390*
Totter, J. R., 76, *92*, 120–122, 126 (see Day), 135 (see Mallory), 138, 150, 151, 155, *158–160*
Tower, S. S., 110, *118*
Towne, L. E., 326, 328, 332, 359, *388*
Trasoff, A., 425, *431*
Traut, H. F., 341, *390*
Trinder, P., 132, *157*
Tschopp, E., 321, *388*, 412, *430*
Tulgan, J., 423, *431*
Turino, T. R., 370, *384*
Turner, C. W., 260–262, 269–272, 277, 280, 283, 285, 286, 296, 297, 299, 302, 304, 307, *310*, *312–314*, 315, 364, 369, *390*
Turpault, M. P., 346, 349, 367, *390*
Twombly, G. H., 328 (see Taylor), *390*
Tyslowitz, R., 216, *227*, 277–279, 287 (see Astwood), 294, 303 (see Astwood), *308*

U

Uber, F. M., 99, *118*
Uebelhoer, T., 418, *428*
Ulrich, P., 372, *390*
Unna, K., 67 (see Singher), *91*, 183, 192, *195*
Uyei, N., 199, 200, 202, *228*

V

Vandenbelt, J. M., 45 (23), 47 (see Bird), *85*
VanderLaan, W. P., 281, 289, 290, 303–305, *308*, *314*, 316
van der Woerd, L. A., 235, 264, *272*
van Dorp, D. A., 27, 28, 34, *36*, *38*
van Dyke, H. B., 198, 201, *229*, *230*
van Eekelen, M., 15, *36*
van Ess, P. R., 28, *36*
van Wagenen, G., 208, *230*, 238, 253, 255, *272*, 365, *390*
Van Winkle, W., Jr., 305, *314*
Vargas, L., 346 (see Lipschütz), *387*
Varney, R. F., 397, *431*
Vasquez-Lopez, E., 249, *272*
Venning, E. H., 414, *428*
Verbrugge, F., 99, *118*
Vermeulen, C., 396, 408, 409, *431*
Vest, S. A., 324, *390*, 394, 413, *431*
Vest, S. A., Jr., 419, *431*
Vetter, H., 99 (see Kuhn), *117*
Vieger, E. A., 45, *92*
Vieira, L. G., 288, *308*
Villada, R. F., 126, *160*
Villela, E., 254, *270*
Vilter, C. F., 123, 140, 142–146, 148, 150, 154 (see Frommeyer; Spies), *158*, *160*, *161*
Vilter, R., 139 (see Moore), 140 (see Moore), *159*
Vilter, R. W., 139, 150, *161*
Vinson, L. J., 191, *194*
Virtanen, A. I., 59, *88*
Vogel, M., 285 (see McGavack), 296–299, 305 (see McGavack), *312*, *314*, 316
van Cappellen, D., 425, *431*
Vonder Heide, E. C., 122, 150, *160*
Von Konrád, E., 370, *390*
Vorzimmer, J., 298, *309*

W

Waddell, J., 168 (see Patton), *173*
Wade, N. J., 78, *87*, 239, *272*
Wadehn, F., 212, *230*
Wagenseil, F., 407, *431*
Wagner-Jauregg, T., 99 (see Kuhn), *117*, 277, *314*
Wahlin, J. G., 140 (see Day), 154 (see Day), *158*
Waisman, H. A., 154, *161*, 164 (67), *173*
Wald, M. H., 275, *308*
Waldman, S., 423, *432*
Walker, H. A., 58 (see Russell), *91*
Walker, O., 30 (see Karrer), *37*
Walker, R., 66, *92*
Walker, T. C., 422, *432*
Wallace, A. W., 423, *431*
Wallace, C., 240, *272*
Wallenfels, K., 6, *37*
Wallen-Lawrence, Z., 201, *230*
Waller, C. W., 119 (see Angier), 121 (see Angier), *157*
Waller, R. K., 285, *314*
Wallich, R., 370 (see Portes), *388*
Walsh, J. W., 371, *390*
Walter, R. I., 327 (see Salmon), 329 (see Geist; Salmon), 332 (see Salmon), 343 (see Salmon), 346 (see Salmon), 354 (see Salmon), 360 (see Geist), *385*, *389*
Waltman, C. L., 365, *390*
Wangensteen, O. H., 418, *432*
Warburg, E., 62, *86*
Warden, H. F., 122, 138, *159*
Warner, E. D., 167 (see Stamler), *173*
Warner, R. C., 63 (see Borsook), *85*
Warren, C. O., 298, *314*
Warren, M. R., 300, *314*
Waterman, L., 264, *272*
Watkins, O., 211, *230*
Watson, C. J., 122, *161*
Watson, H., *391*
Watson, J., 139, 141, *161*
Watson, W. G., 352 (see Child; Woodbury), *384*, *391*
Way, E. L., 191 (see Snell), *195*
Webster, B., 275, 287, *308*, *314*
Webster, B. J., 418, *432*
Webster, E. T., 1 (see Heilbron), *36*

Weichert, C. K., 244, 264, *272*
Weidmann, S., 96, *118*
Weil, L., 213, *230*
Weil, P. G., 374, *390*
Weinglass, A. R., 277, 278, 280, 284, 295–298, 302, *314*, 376 (see Williams), 378 (see Williams), *391*
Weisblat, D. I., 16 (see Hanze), 20 (see Hanze), *36*
Weisler, L., 35 (see Cawley), *38*
Weiss, Z., 4 (see Milas), 9 (see Milas), 15 (see Milas), 16 (see Milas), 18 (see Milas), 19 (see Milas), 22 (see Milas), *37*, *38*
Welch, A. D., 47 (see Wright), 49, 61, 72, 77, *92*, 122, 123 (see Moore), 125 (see Moore), 133 (see Moore), 136, 138, 140, 141 (see Moore), 142 see (Moore), 143 (see Moore), 144, 145 (see Moore), 146–148, 149 (see Moore), 150, 151, 155, 156 (see Wright), *158–161*
Wells, H. B., 221, *230*
Wells, L. J., 238, *269*
Wells, L. T., 253, 255, *272*
Went, F. W., 32 (see Zechmeister), *38*
Went, L. N., 99, *118*
Werbin, H., 43, *91*
Werner, A. A., 409, *432*
Werner, H. W., 300, *314*
Werner, S. C., 376, *390*, 397, *432*
West, R., 376, *390*
Westenbrink, H. G. K., 106, 109, *118*
Westerfeld, W. W., 250, *272*, 290, *314*
Weswig, P. H., 66, *92*
Wettstein, A., 321, *388*
Whipple, D. V., 67, *92*
White, A., 198, 215, 216, 218, 220, 225, 226, *228*, *230*, *231*, 364, *390*
White, A. G. C., 192, *195*
White, E. A., 189, *195*
Whitehead, V. I. E., 299, *311*
Whittenberger, J. L., 376, 378 (see Williams), *391*
Wiedenbauer, F., 106, *118*
Wieder, S., 121, *161*
Wiemer, W., 32, *38*
Wilbrandt, W., 95 (see v. Muralt), *117*
Wilcox, E. A., 324, 346, 351, 362, *386*
Wild, F., 2, *37*
Wilder, O. H. M., 164 (53), 165 (see Record), *173*
Wilder, R. M., 111, *118*
Wilgus, H. S., Jr., 164 (46), 166 (see Heuser), *172*, *173*, 276, 287, *314*
Wilkens, W. E., 213, *231*
Wilkins, L., 321–323, 377, *390*, *391*, 412, *429*
Wilkinson, J. F., 143, 145, 146, 148, *161*
Williams, G. A., 348, *391*
Williams, N. E., 15, *36*
Williams, R. D., 111, *118*
Williams, R. H., 277, 278, 280, 283–285, 290, 291, 295–299, 301–305, *310*, *314*, 316, 376, 378, *391*
Williams, R. J., 72 (see Spies), 74 (see György), 76 (see Mitchell), *87*, *89*, *91*, 120, *159*
Williams, R. R., 84, *92*
Williams, V. R., 45, *92*
Williams, W. I., 45 (10, 11), 46 (see Atkin), 72 (see Atkin), *85*
Williamson, A., 49, 50 (see Parsons), 64, 66 (see Parsons), *90*, *92*
Williamson, J. W., Jr., 300, *314*
Williamson, M. B., 293 (see György), *310*
Wills, L., 125, 139, 141, 152, *161*
Wilson, A., 316
Wilson, H. E., 121 (see Saslaw), 123, 143, 146, 149, *158*, *160*
Wilson, J. A., 394, *431*
Wilson, K., 45 (30), 47 (see Burkholder), *85*
Wilson, L., 331, 348, 351–353, *391*
Wilson, R. H., 60, *92*
Wilson, W. H., 423, *429*
Winkelmann, E., 28 (see Ziegler), *38*
Winkler, A. W., 285, *309*
Winstein, S., 33, *38*
Wintrobe, M. M., 70, *92*, 124, 139, 141, 150, *161*
Wise, E. C., 16 (see Hanze), 20 (see Hanze), *36*
Wittig, A., 32, *38*
Wohlers, H. C., 2 (see Milas), 4 (see Milas), *37*
Wolfe, J. M., 248, 249, 265, *272*
Wolff, J., 287–289, *314*
Wolff, L. K., 15 (see van Eekelen), *36*

Wolff, M., 427
Wollner, A., 350, *391*
Wolter, J. G., 122, *160*
Womack, M., 191, *195*
Woodard, H. Q., 333, 372, *385*, *386*
Woodbury, R. A., 352, *384*, *386*, *391*
Woodward, C. F., 83 (see Badgett), *85*
Woolley, D. W., 41, 46, 65, 71, 74, 75, 77, 82, *88*, *90*, *92*, 185, 189, 191–193, *195*, 316
Woolpert, O. C., 121 (see Saslow), *160*
Wright, A. W., 249, *272*
Wright, C. A., 419, 421, 422, *429*, *432*
Wright, C.-S., 123, 143, 146, 149, *158*
Wright, H. F., 2 (see Milas), 4 (see Milas), 9 (see Milas), 15 (see Milas), 16 (see Milas), 18 (see Milas), 19 (see Milas), 22 (see Milas), *37*, *38*
Wright, H. R., 27 (see Heilbron), *36*
Wright, L. D., 45 (248, 291), 47, 61, 72, *91*, *92*, 122 (see Moore), 123 (see Moore), 125 (see Moore), 133 (see Moore), 141 (see Moore), 142 (see Moore), 143 (see Moore), 144 (see Moore), 145 (see Moore), 146 (see Moore), 148 (see Moore), 149 (see Moore), 156, *159*, *161*
Wright, M., 145, 146, *157*
Wyatt, J., 350, *391*
Wyman, R., 324 (see Shimkin), *389*
Wyss, A., 96, 99, 111, *118*
Wyss, F., 96, 99, 106, 109, 111, *117*, *118*

Y

Yamins, J. L., 9 (see Milas), 15 (see Milas), 16 (see Milas), 18 (see Milas), 19 (see Milas), 22 (see Milas), 25, *38*
Yanine, D., 265 (see Lipschütz), *270*
Youmans, J. B., 51 (see Denko), 72 (see Denko), 74 (see Denko), 75 (see Denko), 77 (see Denko), *86*, 139, *161*
Young, F. G., 215, *231*, 247, *269*
Young, N. F., 337, *387*
Young, R. M., 63, *92*
Young, W. G., 33, *38*

Z

Zechmeister, L., 9, 10, 18, 32, *36*, *38*, 42, *86*, *90*, *92*
Zeithaml, C. E., 418, *432*
Zelson, C., 418, *432*
Zemp, J., 96, 106, *117*
Ziegler, K., 27, 28, *38*
Zondek, B., 235, 244, 247, 248, *272*, 412, *432*
Zubris, A., 13, *36*
Zuckerman, S., 238, 251–253, 255–258, 262, 265, *269*, *271*, *272*, 333, 335, 339, 365 (see Folley), *385*, *391*, 425, *432*
Zuelzer, W. W., 123, 153, *161*
Zwemer, R. L., 225, 227, *229*, *231*
Zwilling, E., 359, *387*

Subject Index

A

Absorption, gastrointestinal, impaired, in sprue, 124
Absorption spectra (see Spectra)
2-Acetylaminofluorene, tumors arising from, 279
Acetylcholine,
  effect of thiamine and thiamine derivatives on, 96, 109, 113
  liberation of, in nerve excitation, 94–96
  relation thiamine content in nerves, 109
3-Acetylpyridine, effect on mice, 192
Achromotrichia, 184
Acne, effect of androgens on, 334
Acromegaly,
  effect of androgens on, 376, 377
    estrogens on, 376, 377
Addison's disease, use of androgens in, 377, 378
Adrenal cortex, source of androgens, 222
Adrenal glands,
  effect of androgens on, 267
    estrogens on, 262–263
    goitrogenic agents on, 295
  hypertrophy of, and estrogens, 263
Adrenalectomy, effect of growth hormone in, 208
Adrenals,
  corticotropic hormone and ascorbic acid content of, 226*ff*.
  corticotropic hormone and cholesterol content of, 226*ff*.
Adrenocorticotropic hormone (see Corticotropic hormone)
Adsorbents, effect of, on vitamin B absorption, 65
Allylthiourea,
  effect on cytochrome oxidase, 291
    muscle, 299
  goitrogenic activity of, 275
  as inhibitor of thyroxine formation, 289
  tumors arising from administration of, 279
Alopecia,
  due to inositol deficiency, 189
    pantothenic acid deficiency, 189
    pyridoxine deficiency, 186
Amino acids, aromatic, effect on cytochrome oxidase, 291
Aminobenzene,
  effect on thyroid, 277
    thyroxine formation, 289
Aminobenzene derivatives, effect on iodine, 287, 300
  goitrogenic activity of, 277
  relative activities of, 303
p-Aminobenzoic acid, antithyroid activity of, 315
  bile salts and, 75
  biological synthesis of, 72
  effect on basal metabolic rate, 284
    melanin formation, 292
    thyroid, 281
    thyroxine formation, 289
  relationship with inositol, 78
  requirement of chick, 168
p-Aminophenylacetic acid, thyroxine formation and, 288
Amphibians, effect of goitrogenic agents on metamorphosis of, 315
"Androgen-estrogen ratio," 420, 421
Androgens, adrenal cortex as source of, 222
  bioassay of, 319–330
  biological effects in women, 325–339
  clinical use of, 339–382
  contraindications, 417
  criteria for bioassay, 320–325
  effect on breast, 334, 367–373
    corpus luteum formation, 327
    diseases aggravated by menstruation, 380
    diseases with high incidence in women, 380
    estrogen excretion, 328
    fallopian tubes, 331
    Graafian follicle, 328
    menstruation, 333
    myometrium, 330, 331
    ovary, 327, 328

pituitary, 326*ff.*
uterus, 329–331
vagina, 331–333
excretion of, 235, 395
homosexuality and, 418–422
inhibitory action on estrogens, 266
interaction with estrogens, 235, 263–267
lactation and, 369
metabolic effects of, 338
modes of administration, 319–325, 411–416
psychological effects of, 339
subjective effects of, 359
synergistic action with estrogens, 266, 267
therapeutic value in angina pectoris, 422–425
in hypogonadism, 411–416
vascular effects of, 338
virilizing effects of, 334
Androsterone,
inhibitory effect on estrogens, 264
interaction with estrone, 267
Anemia,
in children fed goats' milk, 154
macrocytic, of pregnancy, response to pteroylglutamic acid, 141
nutritional macrocytic,
effect of pteroylglutamates on, 122
vitamin M on, 139*ff.*
similarity to vitamin M deficiency, 140
sprue and, 139*ff.*
symptoms of, 13
pernicious,
effect of thymine on, 145, 154
pathogenesis of, 150
of pregnancy, 140
sprue and, 141
therapeutic value of liver extract in, 142, 153
pteroylglutamates in, 122–124, 142–150, 153
pteroylglutamic acid deficiency in, 152
refractory, pteroylglutamic acid and, 121
temporary pernicious, of infancy, criteria of, 153
nutritional origin of, 153
Angina pectoris, androgen therapy of, 422–425
Antibody titers, corticotropic hormone and, 226
Antimony trichloride, determination of vitamin A with, 43
Antithyroid agents (see also Goitrogenic agents)
biology of, 274–307
Antivitamins, 82
in natural foods, 71
and vitamin deficiencies, 71
Arginase, effect of corticotropic hormone on, 222
Ariboflavinosis in mice, 180
symptoms of, 181
Arteries,
effect of ovarian hormone on spiral, 339
of endometrium, 339
Ascorbic acid,
effect of corticotropic hormone on, content of adrenals, 226*ff.*
on cytochrome oxidase, 291
excretion of, 52, 63
requirement of mice, 189, 190
tyrosine and, deficiency, 81
Ascorbic acid oxidase, 62
Atmospheric pressure, corticotropic hormone and resistance to low, 225
Avitaminosis, effect on nerve tissue, 114
Azoöspermia, 410

B

Bile salts, effect of, on availability of vitamin A, 58
Biotin,
biological synthesis, 72
requirement of chicks, 167, 170, 171
of mice, 188
of turkey, 169
sulfonamides and, 74
Biotin-avidin complex, 74
Birds, effect of goitrogenic agents on, 280, 281, 285
Bladder, effect of androgens on, 337, 426
Blood, effect of goitrogenic agents on, 297, 298
Bone marrow, action of goitrogenic agents on, 298

Bone regeneration, phosphatase and, 213
Bradycardia, avitaminosis and, 111
Brain,
  thiamine content of, 106–108
  thiamine deficiency and, hemorrhages, 179
Brassica seeds,
  adrenocortical hypertrophy caused by, 295
  effect of, diet on maturation of ovary, 296
  goitrogenic activity of, 276
Brassica species, goitrogenic activity of, 276
Breast (see also Mammary gland),
  adenofibrosis of, 366
  carcinoma of, 371, 372
  effects of androgens on, 334, 372
  functional and pathological disorders of, 364–373
  pathophysiology of, 364

C

Carbohydrates,
  effect of corticotropic hormone on metabolism of, 224, 225
  intake of, and urinary output of thiamine, 64
  refection and, 66
  synthesis of riboflavin and, 68
Carotenoids, cis-trans isomerism in, 9, 32
Cartilages, effect of growth hormone on epiphyseal, of tibia, 213, 214
Castrates, gonadotropin titer of, 408
Castration,
  effect on pituitary, 250, 326
  gonadotropins and, 398, 408
  homosexuality and, 422
Cats, effect of estrogens on, 258
Celiac disease, 58
  response to pteroylglutamic acid, 153
Chick,
  p-aminobenzoic acid requirement, 168
  biotin requirement, 167, 170, 171
  choline requirement, 167, 168, 170, 171
  folic acid requirement, 168, 171
  inositol requirement, 168
  niacin requirement, 167, 170, 171
  pantothenic acid requirement, 166, 167, 170, 171
  pyridoxine requirement, 167, 170, 171
  riboflavin requirement, 166, 170, 171
  thiamine requirement, 166, 170, 171
  vitamin A requirement, 164, 165, 170, 171
    D requirement, 164, 165, 166, 170, 171
    E requirement, 168
    K requirement, 167, 171
  vitamin content of synthetic diets for the, 170
Chicken, vitamin requirements of mature, 169
Child, androgens and the preadolescent, 417
Cholesterol,
  as precursor of adrenal steroids, 227
  effect of corticotropic hormone on, content of adrenals, 226*ff.*
Choline, 78, 79
  acetylation of, 115
  availability of, 78
  requirement of chick, 170, 171
    of mice, 189
Chondrogenesis, effect of growth hormone on, 200
Climacteric,
  gonadotropins and, 398, 408
  male, 408
    testosterone therapy in, 419
    urinary gonadotropin titers in, 408, 409
Clitoris, effect of androgens on, 335
Cocarboxylase,
  action on heart, 113
  catalytic action on pyruvic acid cycle, 115
Cold, corticotropic hormone and resistance to, 225
Corpus luteum,
  androgens and formation of, 327
  effect of extract of, on the male mammary gland, 260
Creatinuria, effect of androgens on, 379
Cryptorchidism, 417, 418
  due to estrogen injection, 243
  treatment of, 418
Cushing's syndrome,

- effect of androgens on, 374–376
    - estradiol benzoate on, 375
- Cyanides, inhibitory action on thyroxine formation, 289
- Cysteine,
  - effect of goitrogenic agents on, 291
    - on cytochrome oxidase, 291
      - succinoxidase, 291
  - iodine reduction by, 293

D

- Dehydroascorbic acid, 43
- 5-Dehydrohomovitamin A, ethyl ether, 5
  - absorption spectrum of, 9
  - conversion into vitamin A ethyl ethers, 20
- 5-Dehydrovitamin A, ethers, 9–22
  - biological activity, 14
  - synthesis, 9*ff*.
  - ultraviolet absorption spectra, 20
- Dermatosis, pantothenic acid deficiency and, 184
- Development, postnatal, effect of estrogens on, 242–248
- Diabetes,
  - anterior pituitary and, 213
  - corticotropic hormone and, 224, 225
  - growth hormone and alloxan-induced, 213
  - nitrogen retention and, 212
- Diarrhea,
  - response of chronic, to pteroylglutamic acid, 154
  - in sprue, 124
- Dichroism,
  - effect of electric excitation on, 101
  - in living nerve fibres, 101
- Diethylstilbestrol, activity of, 234
- Diiodotyrosine,
  - effect on thyroid, 276
  - factors influencing formation of, 291
- Dimethylaminovitamin A, conversion to vitamin A, 25–27
- Drosophila, action of thiourea on, 283
- Dysentery, nature of, 154
- Dysmenorrhea, 351–356
  - pathophysiology of, 351
  - therapy with androgens, 353
- Dystrophy, adiposogenital, 417
  - muscular, avitaminosis E and, 191

E

- Ejaculate, effect of estrogens on, 240, 241
- Endocervicitis, effect of androgens on, 350
- Endometriosis, 347–349
  - pathophysiology of, 347
  - therapy of, 347*ff*.
- Endometrium,
  - androgens and hyperplasia of, 329*ff*.
  - arteries of, 339
  - histology of, 341
- Enzymes, goitrogenic agents and, 290–293
- Epithelium, effect of estrogens on, of reproductive organs, 258
- Erythrocytes,
  - action of pteroylglutamic acid on level of, 148
    - on maturation of, 144
  - regeneration of, 147
  - response of, to pteroylglutamic acid in pernicious anemia, 147–149
- Estradiol,
  - action on cat, 258
  - activity of α-, benzoate, 234
  - effect on Cushing's syndrome, 375
- Estriol, activity of, 234
- Estrogens,
  - activity of urinary, and hypogonadism, 396
  - antagonism to endogenous androgens, 241
  - chemical structure of, 234
  - combined action of progesterone and, on mammary gland, 261
  - dosage levels of, 261, 262
  - effect on acromegaly, 376, 377
    - accessory sex organs, 251–262
    - adrenals, 262, 263
    - adult males, 236–241
    - androgen excretion, 328
    - body growth, 247
    - castrated mice and rats, 255
    - epithelia, 258
    - external genitalia, 238
    - immature males, 241–248
    - internal genitalia, 239, 240
    - postnatal development, 242–248
    - skin, 238

effects of, compared with hypophysectomy, 246
endogenous, 234, 235
excretion of, 235
interaction with androgens, 235, 266, 267
myotrophic activity of, 251*ff.*
protection of males from effects of, 263–266
recovery from effects of, after cessation of treatment, 247
in young at birth, 235
Estrone,
activity of, 234
interaction with androsterone, 267
Ethyluracil, therapeutic value of, 305
Eunuchoidism,
gonadotropins and, 398
hypogonadotropic, 407
testes in hypogonadotropic, 402
Excretion, urinary,
of vitamin C, 52, 63
of water-soluble vitamins, 52
Extrinsic factor,
in nutritional macrocytic anemia, 139, 141
pernicious anemia, 141
lack of, in temporary pernicious anemia of infancy, 53

F

Factor U, 120
Fallopian tubes, effects of androgens on, 331
Fat,
dietary, and availability of vitamins, 58
effects of androgens on deposition of, 267
estrogens on deposition of, 267
fecal, in sprue, 132, 136
Fatty acids, vitamin B_6 and metabolism of unsaturated, 73
Feminization, of male, effect of estrogens on, 241
Fermentation factor, 121, 138
Fermentation *L. casei* factor, 57
response to, in pernicious anemia, 151
Fern-poisoning, 65
Fertility, effect of testosterone on, 416
Fish, effect on vitamin B absorption, 65
Fluorescence of single nerve fiber, 105
Folic acid (see also Pteroylglutamic acid), 75*ff.*, 121
biological synthesis, 72
conjugates of, 75, 76
and macrocytic anemia, 150
"potential," 155
requirement of chick, 168, 171
of mice, 188
of turkey, 170
therapeutic effect in sprue, 154
use in blood dyscrasias, 298
Foodstuffs, vitamin M content of, 155
Fowl, effect of goitrogenic agents on, 315
Fröhlich's syndrome, 417

G

Genital tract, malignancy of, 350
Genitalia,
effects of estrogens on external, 238
on internal, 239, 240
Glaucoma piriformis, reaction to thiamine, 94
Glossitis, in sprue, 124
Glucoascorbic acid, effect on mice, 189
Glutathione, 63
compared with thiouracil, 293
effect on succinoxidase, 291
iodine reduced by, 293
Glycosuria, corticotropic hormone and, 224, 225
Goitrogenic agents,
action mechanism of, 287–299
activity of, in plants, 275
chemical nature of, 303–305
clinical application of, 305, 306
dietary effects on activity of, 300
direct effects of, on thyroid, 295
distribution in body, 301
effect of, during ontogeny, 283
temperature on activity of, 300
thyroid substance on, 277
on adrenal glands, 295
amphibians, 281, 282, 286, 315
basal metabolism, 277
birds, 280, 281, 285
body growth, 283–287

        bone marrow, 298
        cytochrome oxidase, 291
        fishes, 282, 286
        fowls, 315
        invertebrates, 283
        iodine metabolism, 288
        proteolytic enzymes, 292
        reproductive organs, 296
        reptiles, 281
        thyroid hormone synthesis, 293
    hypophysectomy and, 276, 294
    mammary passage of, 283
    metabolism of, 301–303
    modes of administration, 300*ff*.
    organ lesions due to, 298
    placental transmission, 283
    plants containing, 276
    relation between activity and oxidizability of, 293
    reversibility of activity, 279
    sex differences in response to, 299
    species differences in resistance to, 301
    thyroidectomy and, 285, 286
    toxic reactions to, 306
Gonadotropins,
    in castrates, 408
    hypogonadism and urinary, 397
Gonads, effect of corticotropic hormone on, 222, 223
Graafian follicle, effect of androgens on, 328
Granulocytopenia, caused by thiouracil, 298
Graves' disease,
    effect of androgens on, 378
    therapeutic value of goitrogenic agents, 305
Ground squirrel, effect of estrogens on, 255
Growth,
    effect of estrogens on, 243, 246
    growth hormone and continuous, 200, 208
    hypophysectomy and, rate, 200, 246
    inhibitory effect of corticotropic hormone on, 221, 222
    relative, rates of mice and rats, 175, 176
Growth hormone (see Pituitary, growth hormone)
Guanidine carbonate, goitrogenic activity of, 277
Gynecomastia, 407

H

Hair
    effect of estrogens on growth of, 238
    pantothenic acid deficiency and graying of, 183
Heart, action of goitrogenic agents on, 285
Hernia arising from estrogens, 236
Hexestrol, activity of, 234
Homosexuality,
    androgens and, 418–422
    hormonal imbalance and, 421
Homovitamin A ethyl ether, 8
    absorption spectrum of, 9
Hormone
    corticotropic
        analysis of, 220
        biological properties, 221–226
        diabetogenic action, 224
        effect on carbohydrate metabolism, 224
            cartilages, 223
            hypophysectomized rats, 215
            liver arginase, 222
            lymphoid tissues, 225
            osseous system, 221
            seminal vesicle, 224
            serum proteins, 225
        growth hormone and, 221, 222
        isoelectric point of, 220
        isolation of, 216–219
        methods of assay of, 215, 216
        physical and chemical properties, 220, 221
        resistance to cold and, 225
            to low atmospheric pressure, 225
        solubility of, 220
        stability of, 220
    follicle-stimulating, urinary excretion, 326
    gonadotropic,
    estrogens and, 250, 251
    hypogonadism and, 398
17-Hydroxycorticosterone and glycosuria, 225

Hyperestrogenemia, premenstrual, 356
Hyperthyroidism, thiamine and, 64
Hypertrichosis, effect of androgens on, 335
Hypogonadism, 394–411
  clinical features, 400–406
  definition of, 394
  detection of, 394
  hypergonadotropic, 406, 408
  hypogonadotropic, 401, 402
  pathology of, 398–400
Hypophysectomy,
  effect of estrogens following, 260, 261
    on goitrogenic activity, 277
      growth rate, 246
  growth hormone and, 208
Hypoprothrombenia, effect of pteroylglutamic acid on, 134

I

Impotence, 418, 419
  effect of testosterone on, 419
Infantilism, 406
Invertebrates, effect of goitrogenic agents on, 283
Iodide, effect on goitrogenic agents, 276
Iodine,
  effect of goitrogenic agents on metabolism of, 287–290
    on pituitary, 279
      thyroid, 275
  goitrogenic activity and, 287–289
  radioactive, 288, 289
  reaction with thioureas, 315
  reduction by p-aminobenzoic acid, 315
    thiouracil, 315
    thiourea, 293, 315
  retention in thyroid, 289
  thyroid enlargement and, 275
  thyroxine formation and, 289
  uptake by thyroid, 288, 289
  xanthine oxidase and, 291
Inositol, 74, 77*ff.*
  interrelations with p-aminobenzoic acid, 78
  microorganisms and synthesis of, 77
  requirement of chick, 168
    of mice, 111
Intestinal synthesis of vitamins, 50
Intrinsic factor, in pernicious anemia, 141
β-Ionone, 2
β-Ionylideneacetaldehyde, 1, 2
Isobutylthiouracil, therapeutic value of, 305
Isothiourea derivatives, adrenaline and, 296

K

17-Ketosteroids, urinary,
  effect of androgens on, 396
  hypogonadism and, 396
Kidney,
  effect of androgens on, 336
    goitrogenic agents on, 297
"Klinefelter's syndrome," 410, 427

L

L. casei factor (see also Folic acid), 120, 121
  of liver, 121
  urinary excretion of, in pernicious anemia, 151
Lactation,
  effect of estrogens on, 261
  inhibitory effect of androgens on, 370
  ovarian transplants and, in male guinea pigs, 259
Lecithin, effect on availability of vitamin A, 58
Leucopenia,
  caused by thiouracil, 298
  effect of pteroylglutamic acid on, 216
  in sprue, 124
Leydig cells, 404
  failure of, 400
  testosterone and failure of, 419
Libido,
  effect of androgens on, 362
  pathophysiology of, 361
Light, ultraviolet,
  absorption by thiamine, 103
  action on vagus perfusion liquid, 112
  effect on nerve excitability, 102
Liver,
  effect of goitrogenic agents on, 294
  extract, therapeutic effect in pernicious anemia, 142

  glycogen, effect of α-naphthylthiourea on, 297
Lupus erythematosus, androgens and, 381
Lymphocytes, corticotropic hormone and, 226
Lymphoid tissues, corticotropic hormone and, 225
Lymphopenia, corticotropic hormone and, 226

M

Macrocytosis, reduction after pteroylglutamic acid therapy, 149
Malnutrition and vitamin availability, 79
Mammary gland (see also Breast),
  action of androgens on, 364
  effect of corpus luteum extract on the male, 260
    estrogens on, 259–262
Menopausal syndrome,
  pathophysiology of, 358
  therapeutic effect of androgens on, 358
Menorrhagia, 359
  therapy with androgens, 343
Menstruation,
  diseases aggravated by, 380
  effect of androgens on, 333
  pathophysiology of, 339–341
Mercaptothiazoline, modes of administration, 301
Metabolism,
  basal, effect of goitrogenic agents on, 275, 284
    methylthiouracil on, 284
    sulfaguanidine on, 284
    thiouracil on, 284
    thyrotropin on, 284
    thyroxine on, 293
  growth-promoting extract and, 212
Metamorphosis, amphibian, inhibitory effect of thiouracil on, 282
    thiourea on, 282
Methyl cyanide, goitrogenic activity of, 276
Methyltestosterone,
  clinical use of, 339–381
  modes of administration, 411–416
  relative activity, 319*ff.*, 339*ff.*
  therapeutic value in hypogonadism, 411*ff.*
Methylthiouracil,
  effect on basal metabolic rate, 284
    thyroid, 280*ff.*
  toxicity of, 316
Mice,
  effect of riboflavin on, 181
  relative growth rates of, 175, 176
  thiamine deficiency in, 176, 177
  vitamin requirements of, 175–194
Monoiodoacetic acid,
  as a nerve poison, 99
  thiamine accumulation in nerve and, 115
Moulting, effect of thiourea on, 282
Müllerian tissue, response of, to estrogens, 258
Muscle,
  androgens and dystrophy of, 379, 380
  estrogens and development of smooth, 251*ff.*
  thiamine deficiency and lesions of, 178
    and necrosis of, 179
  vitamin E and dystrophy of, 152
    paralysis of, 191
Myoma,
  effect of androgens on, 346
  pathophysiology of, 346
Myometrium,
  effect of androgens on, 330, 331
  role of, in functional uterine bleeding, 341

N

α-Naphthylthiourea, action on liver glycogen, 297
α-Naphthylurea,
  effect on rats, 315
    dogs, 315
"Necrosin," 341
Neocryptoxanthin A, provitamin A activity of, 32
Neoplasms, thyroid, arising from treatment with antithyroid substances, 306, 315
Neovitamin A, 32, 33
  biological potency of, 33
Nerve fibers,
  effect of electric excitation on living, 101

fluorescence of single, 105
ultraviolet absorption of, 99
Nerves,
effect of avitaminosis on, 114
ultraviolet light on excitability of, 102
liberation of acetylcholine in excitation of, 94–96
thiamine in, 96–105, 114
monoiodoacetic acid poisoning of, 99
thiamine content of, 103, 105–111
Niacin,
derivative of, in cereal products, 83
low protein diet and deficiency, 70
requirement of chicks, 167, 170, 171
mice, 189
turkey, 169, 170, 171
response of yeast to, 47
synthesis of, by intestinal flora, 70, 71
in rat, 69
Nitriles, goitrogenic activity of, 275
Nitrogen,
alloxan-induced diabetes and retention of, 212
growth hormone and, 211, 212
Norit eluate factor, 120

O

Oil, effect on carotene absorption, 59
Orchiectomy, urinary gonadotropins and, 406
Organs, reproductive, response of epithelium of, to estrogens, 258
Osseous system, effect of corticotropic hormone on, 221, 222
Osteogenesis, growth hormone and, 200
Osteoporosis, postmenopausal, 376
Otosclerosis, effect of androgens on, 381
Ovary, effect of androgens on, 327
Oxidation-reduction potentials in thyroid, 292

P

Pancreas, cystic fibrosis of, 58
Panhypopituitarism, gonadotropins and, 398
Pantothenic acid,
biological synthesis, 72
body weight of mice and, 182
requirement of chick, 166, 167, 170, 171
mice, 182
turkey, 170
response of microorganisms to, 46
Pantoyltaurine, effect on mice, 191
Paralysis,
partial, in mice due to pantothenic acid deficiency, 183
to pyridoxine deficiency, 185
Pelvic inflammatory disease, 349
"Percorten," action on heart, 113
Peroxidase, effect of goitrogenic agents on, 290
Phenylthiourea, goitrogenic activity of, 276
Phosphatase,
bone regeneration and, 213
goitrogenic agents and, 292
response to vitamin D and, 60
Pigeon, vitamin requirement of, 169
Pituitary,
diabetogenic activity of, 245
effect of androgens on, 265–267, 357
castration on, 250
estrogens on, 243–246, 248–251, 265–267
estrogen response and, 261
goitrogenic agents and, 276, 279*ff.*, 315
anterior,
diabetogenic action of, 213
effect of androgens on, 326–329
castration on, 326
hormones of, 198
growth hormone,
assay of, 198–215
biological properties of, 208–215
corticotropic hormone and, 221, 222,
diffusion of, 205
effect of pH on activity of, 207
on chondrogenesis, 213
epiphyseal cartilages of tibia, 213
osteogenesis, 213
electrophoresis of, 233
fractionation of, 203
globulin fraction of, 203
history of, 201, 202
isoelectric point of, 203, 206
isolation of, 201–206
nitrogen retention and, 211, 212

    physical and chemical properties, 206–208
    solubility of, 206
    weight gain and, 212
  luteotropic factor, 329
Plants,
  containing goitrogenic agents, 276
  goitrogenic activity of, 275
Polytomella caeca, reaction to thiamine, 94
Poultry (see also chick), vitamin requirements, 164–171
Pregnancy,
  effect of androgens on nausea and vomiting of, 373
  pernicious anemia of, 140*ff*.
Premenstrual tension,
  pathophysiology of, 356
  therapy with androgens, 357
Progesterone, effect on corpus luteum formation, 355
Prolactin, 218
Promizole,
  direct effects of, on thyroid, 295
  effect on basal metabolic rate, 284
    iodine retention, 288
    pituitary, 280
  reversibility of effect on thyroid, 279
Propylene glycol, as solvent for androgens, 323
Propylthiouracil,
  therapeutic value, 305
  toxicity, 316
Prostate,
  effect of estrogens on, 251
  hypertrophy of, 425–427
    testosterone therapy in, 425
Protein metabolism, vitamin E and, 191
Proteins,
  choline availability and intake of, 78
  feeding of, in testicular deficiency, 414
  serum, corticotropic hormone and, 225
  urinary excretion of riboflavin and intake of, 181
Pruritus,
  use of androgens in senile, 381
  vulvae, use of androgens in, 381
Psychiatric disorders, use of androgens in, 381
Pteroylglutamates, 121, 122, 138
  hemopoietic activity, 122
Pteroylglutamic acid (see also Folic acid), 121–152
  action mechanism of, 151
  clinical response to, in sprue, 125*ff*.
  gastrointestinal absorption and, 131
  hematologic response to, in sprue, 129, 130
  history of, 122*ff*.
  liberation from pteroylheptaglutamate, 151
  modes of administration, 143*ff*.
  relation to anti-pernicious anemia principle, 143
  response of yeast to, 47
  reticulocyte response to, in pernicious anemia, 145
  therapeutic effect in diseases of infants and children, 152–154
    in pernicious anemia, 142–150
  thymine synthesis and, 45
  variability in response to, in pernicious anemia, 150
Pteroylhexaglutamylglutamic acid, 138
Pteroyltriglutamic acid, 121, 122, 138
Pyracin, requirement of chick, 168
Pyridine-3-sulfonic acid, effect on mice, 192
Pyridoxal, growth activity in mice, 187
Pyridoxamine, growth activity in mice, 187
Pyridoxine,
  effect on body weight of mice, 186
  growth activity in mice, 187
  requirement of chick, 167, 170, 171
    of mice, 185–188
    of turkey, 170
  therapeutic value in blood dyscrasias, 298
Pyruvic acid, cocarboxylase as catalyst in, cycle, 115

R

Rape seed,
  effect on thyrotropin content of pituitary, 294
    on thyroid, 276, 281
  goitrogenic activity, 276*ff*.
  thyroid neoplasms arising from prolonged diet of, 306

Rats,
  effect of corticotropic hormone on hypophysectomized, 215
    growth hormone on tibia of hypophysectomized, 200
  "plateaued," 200
  relative growth rates, 175, 176
Refection, 66
Resorption gestation, in mice due to vitamin E deficiency, 190
Reticulocytes,
  response to liver extract, 145
    pteroylglutamic acid, 145
    thymine, 145
Riboflavin,
  absorption of, 69
  carotenoid pigments and, 42
  dietary fat and requirement of, 68
  effect on food utilization in mice, 181
  phenazine analogue of, 192
  requirement of chicks, 166, 170, 171
    mice, 182
  urinary excretion of, and protein intake, 68

S

Salts, effect on vitamin stability, 84
Sea urchin, action of thiourea on, 283
Seminal vesicles,
  effect of corticotropic hormone on, 224
    estrogens on, 251–259
Seminiferous tubule, failure of, 410
Sex differentiation, effect of estrogens on, 241, 242
Sex hormones (see under Androgens, Estrogens, Testosterone, etc.)
Simmond's disease, effect of androgens on, 376
Skeleton, effects of estrogens on, 236
Skin, effects of estrogens on, 238, 381
Sodium azide,
  effect on thyroid hormone formation, 289
  sulfide, effect on thyroxine formation, 289
Spectra, absorption, of homo-vitamin A ethyl ether, 9
  of single nerve fibers, 99
  of vitamin A derivatives, 9
    homologues, 9
Spermatogenesis, 404
  effect of exogenous testosterone on, 415
Sprue, 124–139
  relation to vitamin M deficiency, 125
  response to liver extract, 130
  symptoms of, 124*ff.*
  therapeutic value of pteroylglutamates in, 125, 130*ff.*, 154
    of thymine in, 154
Starvation, compared to thiamine deficiency, 114
Steatorrhea, in sprue, 124
Stereoisomerism, in vitamin A derivatives, 32
Strepogenin, effect on mice, 191
Structure, chemical, correlation between, and antithyroid activity, 316
Succinoxidase, effect of goitrogenic agents on, 291
Sulfa compounds, see Sulfonamides
Sulfadiazine,
  dietary factors affecting activity of, 300
  effect on thyroid, 281, 282
    thyrotropin, 294
  goitrogenic activity of, 276
Sulfaguanidine,
  effect on acetonitrile activity, 293
    basal metabolic rate, 284
    body growth, 283
    food intake, 283
    proteolytic enzymes, 277
    thyroid, 277, 292
  goitrogenic activity of, 276, 315
  response to, 287
  reversibility of effect on thyroid, 279
Sulfanilamide, goitrogenic activity, 276
Sulfanilic acid, goitrogenic activity, 277
Sulfapyridine,
  effect on guinea pig thyroid, 276
  goitrogenic activity of, 276
Sulfathiazole, effect on heart function, 285
Sulfonamides, 286*ff.*
  anemia due to, 297
  atrophy of adrenals due to, 295
  biotin deficiency due to, 74
  cytochrome oxidase and, 291
  dietary factors and activity of, 300
  effect on kidney, 297
    liver, 286, 297

    lung tissue, 298
    thyroxine formation, 289
  inhibition of melanine formation by, 292
  iodine and, 293
  organ lesions caused by, 298
  peroxidase activity and, 290
  succinoxidase and, 291

T

Tails, necrotic, as symptom of pyridoxine deficiency, 186
Testes,
  atrophy of, 410
  biopsy of, 398
  effect of androgens on descent of, 417, 418
  estrogen content of, 235
Testosterone
    effect on estrogens, 264, 267
      seminiferous tubules, 399
      spermatogenesis, 415
    hypogonadism and, 411*ff*.
    modes of administration, 411*ff*.
    relative activity of, 319–325
  propionate,
    clinical use of, 339–381
    effect on endometrium, 329, 330
    modes of administration, 411*ff*.
    relative activity, 319–325
    therapeutic value in hypogonadism, 411*ff*.
Thiaminase, 56
Thiamine,
  absorption of ultraviolet light by, 99
  accumulation in poisoned nerve, 115
  analogues, effect on mice, 192
  content of brain, 106–108
    peripheral nerves, 103, 105–110
  deficiency, effects of, in mice, 176–178
  derivatives, effect on acetylcholine action, 113
  excretion of, 52
  free and bound, 105*ff*.
  hyperthyroidism and, 64
  inhibitory effect on acetylcholine, 96, 109, 113
  liberation by cholinergic nerve, 114
    in nervous excitation, 96–105
    upon vagal stimulation, 111
  photochemical sensitivity, 102
  relation between acetylcholine and, content of nerves, 109
  requirement of chicks, 166, 170, 171
    of mice, 176*ff*.
    of pigeons, 169–171
  response of microorganisms to, 46, 94
  sensitizing action on acetylcholine, 96
  transformation to thiochrome, 104, 105
  ultraviolet absorption, 99
  in yeast, 66
Thiochrome, conversion of thiamine into, 105
Thiocyanates,
  effect on iodine metabolism, 288
  goitrogenic activity of, 275
  inhibitory effect on thyroxine formation, 288
Thiouracil,
  adenoma and, 306
  administration prior to thyroid removal, 306
  adrenotropic hormone and, 295
  autonomic nervous system and, 299
  bone deformities caused by, 286
  effect on amphibian metamorphosis, 282
    basal metabolic rate, 284
    blood cells, 297
    body growth, 284–286
      weight, 285
    bone marrow, 298
    cytochrome oxidase, 291
    fat deposition, 285
    kidney, 297
    liver, 297
    peroxidase activity, 290
    pituitary, 280
    plasma protein, 285
    reproductive system, 296
    serum albumin, 287
    succinoxidase, 291
  glutathione compared with, 293
  goitrogenic activity, 277, 315
  inhibitory action on thyroxine formation, 289
  interaction with acetonitrile, 293
  iodine and, 288, 300

  metabolism of, 301
  periarteritis nodosa due to, 299
  reversibility of effect on thyroid, 279
  sex differences in response to, 299
  therapeutic value in Grave's disease, 305
  thyroid neoplasms caused by, 279
  thyrotropin and, 294, 299
Thiouracil compounds, therapeutic value in hyperthyroidism, 305
Thiourea,
  adenocarcinomas caused by, 279
  derivatives, adrenaline and, 296
    anemia caused by, 297
    effect of dietary factors on activity of, 300
      on melanine formation, 292
    reduction of iodine by, 293
    relative activities of, 303
    thyroid neoplasms caused by, 306, 315
  effect on adrenal cortex, 295
    amphibian metabolism, 281
    basal metabolism, 284
    body growth, 284, 286
    cytochrome oxidase, 291
    fat deposition, 285
    heart function, 285
    iodine metabolism, 287
    liver, 286, 297
    moulting, 282
    peroxidase, 290
    pituitary, 280
    plasma protein, 285
    proteolytic enzymes, 292
    reproductive organs, 296
    secondary sex characteristics, 286
    serum albumin, 287
    succinoxidase, 291
    thyroid, 277, 280, 281, 284, 289, 293, 295
    xanthine oxidase, 291
  goitrogenic activity, 277*ff.*, 315
  hemosiderosis caused by, 299
  interaction with acetonitrile, 293
  iodine and, 288, 300, 315
  mechanism of antithyroid action, 293
  metabolism, 301
  milk production and, 296
  reversibility of effect on thyroid, 279
  therapeutic value in Grave's disease, 305
  thyroid neoplasms arising from, 315
  toxicity of, 283
Thymine, 154, 155
  compared with folic acid, 154
  effect of pleroylglutamic acid on bacterial synthesis of, 45
  therapeutic value in pernicious anemia, 123, 154
    sprue, 154
Thymus gland, effect of corticotropic hormone on, 225
Thyroid,
  adenomata of, 278, 279
  effect of rape seed feeding on, 276
    thiouracil on, 315
    thiourea on, 315
  factors influencing the, 274
  goitrogenic agents and hyperplasia of, 280, 281
  hormone (see also thyroxine) effect of thiourea on secretion of, 292
    formation of, 285
    as regulator of thyrotropin, 293
    relation between temperature and requirement of, 300
  iodine content of, 288, 289
  proteolytic enzymes of, 292
  substance
    effect on goitrogenic activity, 279
      on pituitary changes due to goitrogenic agents, 279
  thyrotropin and hyperplasia of, 294
Thyroidectomy,
  and activity of growth hormone, 208
  thiouracil feeding compared with, 287
Thyrotropin,
  action of goitrogenic agents on, 294, 295
  activation by thiouracil, 295
    of thyroid by, 293
  blood levels of, 294
  effect on amphibian metamorphosis, 282
    basal metabolic rate, 284
    iodine uptake, 288
    thiouracil content of thyroid, 302

factors regulating formation and release of, 293, 294
proteolytic enzymes and, 292
thiouracil compared with, 294
thyroid and, 294
Thyroxine (see also Thyroid hormone)
antagonists of, 316
effect on basal metabolism, 285, 293
iodine accumulation, 288
thyroid, 281
factors influencing formation of, 284, 289*ff*.
inactivation of, 293
inhibitory action on goitrogenic agents, 276, 278
reversal of pituitary changes due to goitrogenic agents by, 280
thiourea and, 286
Tibia, effect of growth hormone on, 200
Tocopherol,
determination, 44
effect on carotene absorption, 59
mice, 193
response to vitamin E, 60
levels in sprue, 133
requirement of mice, 191
dl-α-Tocopherolquinone, effect on mice, 193
Tocopherols, enhancement of vitamin A effect by, 60
Triphenylethylene, effect on testes, 240
Tryptophan and niacin requirement, 81
Tumors, pituitary, caused by estrogens, 248
Turkey, vitamin requirement of, 169*ff*.
Tyrosinase, inhibitary effect of goitrogenic agents on, 292
Tyrosine, and ascorbic acid deficiency, 81

U

Urinary tract, functional disturbances, 350
Uterus,
contractions of, 355
effect of androgens on, 329–331
functional bleeding, therapy with androgens, 339–345
masculinus, effect of estrogens on, 255, 257

V

Vagina, effect of androgens on, 331–333
Vagus,
action of ultraviolet light on, perfusion fluid, 112
liberation of thiamine on stimulation of, 111
"Vagusstoff," 114
Virilism, androgens and, 336
Vitamin determination,
limitations of methods for, 41
microbiological methods, 44
Vitamin A
compared to neovitamin A, 33
configuration 32
conversion of dimethylaminovitamin A to, 25–27
gastrointestinal absorption, 131
homogeneity of, 33
requirement of chicks, 164, 165, 170, 171
mice, 190
spectrophotometric analysis, 42
stereochemistry of, 30
stereoisomers of, 32
synthesis, 1–4
"Vitamin A acid," 27–30
Vitamin A esters, synthesis of, 22–25
Vitamin A ethers, 10–22
biological activity of, 13, 14
synthesis, 9-22
ultraviolet absorption spectra of, 20
Vitamin A homologues,
biological activity of, 4
synthesis of, 4–9
ultraviolet absorption spectra, 9
Vitamin Bc, 120, 121
Vitamin Bc conjugate, 120
response to, in pernicious anemia, 151
Vitamin B_6,
carbohydrates and requirement of, 73
and metabolism of unsaturated fatty acids, 73
response of yeast to, 46, 72
Vitamin C, determination of, 43
Vitamin D,
chemical determination of, 4
requirement of chicks, 164–166, 170, 171

    - mice, 190
    - turkey, 169
- Vitamin E,
  - muscular dystrophy and, 168
  - requirement of chick, 168
    - mice, 190, 191
- Vitamin K,
  - analogues, effect on mice, 193
  - deficiency, effect of pteroylglutamic acid in, 134
  - requirement of chick, 167, 171
- Vitamin M,
  - deficiency,
    - in monkeys, 125
    - related to sprue, 125
    - similarity to nutritional macrocytic anemia, 140
  - nutritional importance, 139, 155*ff.*
  - pernicious anemia and, 151
  - requirement of, 136*ff.*
- Vitamin P, 62
- Vitamin analogues, effect on mice, 191–193
- Vitamin content in synthetic chick diets, 170
- Vitamin deficiencies, antivitamins and, 71
- Vitamins,
  - assay procedures, 47–49
  - availability of, 54, 82, 83
  - biosynthesis, 80*ff.*
  - excretion in urine, 50
  - interrelationship with amino acids, 41
    - vitamins, 40, 71, 72, 81*ff.*
  - requirements of poultry, 164–171
    - mice, 175–194
  - secondary deficiencies of fat-soluble, 124
  - in sweat, 50
- Vocal cords, effect of androgens on, 336
- Vulva, effect of androgens on, 335

W

- Weight,
  - effect of androgens on body, 267
    - growth hormone on, 212
    - estrogens on 131
    - pteroylglutamic acid on gain of, in sprue, 131
  - urinary nitrogen and body, 212

X

- p-Xanthine, antithyroid activity of, 305
- Xanthine oxidase, effect of goitrogenic agents on, 291
- Xanthopterin, activity of, 76
- Xanthurenic acid, excretion in mice, 187
- X-zone, effects of estrogens on, 262

Y

- Yeast fermentation method for thiamine determination, 99

Cumulative Index of Vols. I–V

AUTHOR INDEX

Ansbacher, S., p-Aminobenzoic Acid—Experimental and Clinical Studies. II, 215 (1944)
Bacharach, A. L., see Heilbron, I. M.
Barrett, R., see Najjar, V. A.
Best, C. H., and Lucas, C. C. Choline—Chemistry and Significance as a Dietary Factor. I, 1 (1943)
Bird, H. R., The Vitamin Requirements of Chicks. V, 163 (1947)
Biskind, M. S., Nutritional Therapy of Endocrine Disturbances. V, 147 (1945)
Burk, D., and Winzler, R. J., Vitamins and Cancer. II, 306 (1944)
Carter, A. C., Cohen, E. J., and Shorr, E., The Use of Androgens in Women. V, 317 (1947)
Charipper, H. A., and Gordon, A. S., The Biology of Antithyroid Agents. V, 273 (1947)
Clements, F. W., Manifestations of Nutritional Deficiency in Infants. IV, 73 (1946)
Cohen, E. J., see Carter, A. C.
Cornett, M. L., see McHenry, E. W.
Cox, G. J., A Critique of the Etiology of Dental Caries. II, 255 (1944)
Crowfoot, D., X-Ray Crystallography and Sterol Structure. II, 409 (1944)
Daft, F. S., and Sebrell, W. H., Sulfonamides and Vitamin Deficiencies. III, 49 (1945)
Darby, W. J., The Physiological Effects of the Pteroylglutamates in Man—with Particular Reference to Pteroylglutamic Acid (PGA). V, 119 (1947)
Day, P. L., The Nutritional Requirements of Primates other than Man. II, 71 (1944)
Dodds, E. C., Hormones in Cancer. II, 353 (1944)
Dodds, E. C., Possibilities in the Realm of Synthetic Estrogens. III, 229 (1945)
Elkins, M., see SubbaRow, Y.
Emmens, C. W., see Parkes, A. S.
Emmens, C. W., and Parkes, A. S., Effect of Exogenous Estrogens on the Male Mammal. V, 233 (1947).
Evans, H. M., see Li, C. H.
Gordon, A. S., see Charipper, H. A.
Hall, P. P., Growth Factors for Protozoa. I, 249 (1943)
Hastings, A. B., see SubbaRow, Y.
Heilbron, I. M., Jones, W. E., and Bacharach, A. L., The Chemistry and Physiology of Vitamin A. II, 155 (1944)
Heller, C. G., and Maddock, W. O., The Clinical Uses of Testosterone in the Male. V, 393 (1947)
Hertz, R., Effect of B-Vitamins on the Endocrinological Aspects of Reproduction. IV, 135 (1946)
Hogan, A. G., see Pfiffner, J. J.
Houssay, B. A., The Thyroid and Diabetes. IV, 188 (1946)
Joliffe, N., and Most, R. M., The Appraisal of Nutritional States. I, 60 (1943)
Jones, W. E., see Heilbron, I. M.
Knight, B. C. J. G., Growth Factors in Microbiology—Some Wider Aspects of

Nutritional Studies with Microorganisms. III, 108 (1945)
Kochakian, C. D., The Protein Anabolic Effects of Steroid Hormones. IV, 256 (1946)
Li, C. H., and Evans, H. M., The Properties of the Growth and Adrenocorticotropic Hormones. V, 197 (1947)
Loofbourow, J. R., Physical Methods for the Identification and Assay of Vitamins and Hormones. I, 109 (1943)
Lucas, C. C., see Best, C. H.
Maddock W. O., see Heller, C. G.
Mason, K. E., Physiological Action of Vitamin E and its Homologues. II, 107 (1944)
McHenry E. W., and Cornett, M. L., The Role of Vitamins in the Anabolism of Fats. II, 1 (1944)
Melnick, D., and Oser, B. L., Physiological Availability of the Vitamins. V, 39 (1947)
Melville, D. B., The Chemistry of Biotin. II, 29 (1944)
Milas, N. A., The Synthesis of Vitamin A and Related Products. V, 1 (1947)
Minot, G. R., and Strauss, M. B., Physiology of Anti-Pernicious Anemia Material. I, 269 (1943)
Mitchell, H. H., The Chemical and Physiological Relationship between Vitamins and Amino Acids. I, 157 (1943)
Moore, T., The Interrelation of Vitamins. III, 1 (1945)
Morris, H. P., Vitamin Requirements of the Mouse. V, 175 (1947)
Most, R. M., see Joliffe, N.
von Muralt, A., Thiamine and Peripheral Neurophysiology. V, 93 (1947)
Nachmansohn, D., The Role of Acetylcholine in the Mechanism of Nerve Activity. III, 337 (1945)
Najjar, V. A., and Barrett, R., The Synthesis of B-Vitamins by Intestinal Bacteria. III, 23 (1945)
Oser, B. L., see Melnick, D.
Parkes, A. S., see Emmens, C. W.
Parkes, A. S., and Emmens, C. W., Effect of Androgens and Estrogens on Birds. II, 361 (1944)
Pearlman, W. H., see Pincus, G.
Pfiffner, J. J., and Hogan, A. G., The Newer Hematopoietic Factors of the Vitamin B-Complex. IV, 1 (1946)
Pincus, G., and Pearlman, W. H., The Intermediate Metabolism of the Sex Hormones. I, 294 (1943)
Reichstein, T., and Shoppee, C. W., The Hormones of the Adrenal Cortex. I, 346 (1943)
Reineke, E. P., Thyroactive Iodinated Proteins. IV, 207 (1946)
Schneider, H. A., Nutrition and Resistance to Infection: the Strategic Situation. IV, 35 (1946)
Sebrell, W. H., see Daft, F. S.
Shoppee, C. W., see Reichstein, T.
Shorr, E., see Carter, A. C.
Strauss, M. B., see Minot, G. R.
SubbaRow, Y., Hastings, A. B., and Elkin, M., Chemistry of Anti-Pernicious Anemia Substances of Liver. III, 238 (1945)
Sulman, F., see Zondek, B.
Thayer, S. A., Methods of Bioassay of Animal Hormones. IV, 312 (1946)

WALD, G., The Photoreceptor Function of the Carotenoids and Vitamins A. I, 195 (1943)
WARKANY, J., Manifestations of Prenatal Nutritional Deficiency. III, 73 (1945)
WILLIAMS, R. J., The Significance of the Vitamin Content of Tissues. I, 229 (1943)
WINZLER, R. J., see BURK, D.
ZONDEK, B., AND SULMAN, F., Mechanism of Action and Metabolism of Gonadotropic Hormones in the Organism. III, 297 (1945)

SUBJECT INDEX

Acetylcholine and mechanism of nerve activity (NACHMANSOHN). III, 337 (1945)
Adrenal Cortex, hormones of the (REICHSTEIN). I, 346 (1943)
Adrenocorticotropic hormone, properties (LI). V, 197 (1947)
Amino Acids, chemical and physiological relationship between, and vitamins (MITCHELL). I, 157 (1943)
p-Aminobenzoic Acid (ANSBACHER). II, 215 (1944)
Androgens, effect of, and of estrogens on birds (PARKES). II, 361 (1944)
Androgens, use of, in women (CARTER). V, 315 (1947)
Anti-Pernicious Anemia Substances, physiology of (MINOT). I, 269 (1943)
Antithyroid agents, biology (CHARIPPER). V, 273 (1947)
Bacteria, synthesis of B vitamins by intestinal (NAJJAR). III, 23 (1945)
Biotin, chemistry (MELVILLE). II, 29 (1944)
Birds, effect of androgens and estrogens on (PARKES). II, 361 (1944)
Cancer and vitamins (BURK). II, 306 (1944)
—, hormones in (DODDS). II, 353 (1944)
Caries, dental, etiology (COX). II, 255 (1944)
Carotenoids, photoreceptor function of, and vitamins A (WALD). I, 195 (1943)
Chicks, vitamin requirements (BIRD). V, 163 (1947)
Choline, chemistry and significance as dietary factor (BEST). I, 1 (1943)
Diabetes and thyroid (HOUSSAY). IV, 188 (1946)
Endocrine Disturbances, nutritional therapy (BISKIND). IV, 147 (1946)
Estrogens, effect of, and of androgens on birds (PARKES). II, 361 (1944)
—, —— exogenous, on the male mammal (EMMENS). V, 233 (1947)
—, possibilities of synthetic (DODDS). III, 229 (1945)
Fats, role of vitamins in the anabolism of (McHENRY). II, 1 (1944)
Growth factors for Protozoa (HALL). I, 249 (1943)
—— in microbiology (KNIGHT). III, 108 (1945)
Growth hormone, properties (LI). V, 197 (1947)
Hormones of the adrenal cortex (REICHSTEIN). I, 346 (1943)
—, bioassay of animal (THAYER). IV, 312 (1946)
— in cancer (DODDS). II, 353 (1943)
—, mechanism of action and metabolism of gonadotropic in the organism (ZONDEK). III, 297 (1945)
—, physical methods for identification and assay of vitamins (LOOFBOUROW). I, 109 (1943)
—, protein anabolic effects of steroid (KOCHAKIAN). IV, 256 (1946)
Infection, nutrition and resistance to (SCHNEIDER). IV, 35 (1946)
Liver, chemistry of anti-pernicious anemia substances of (SUBBAROW). III, 248 (1945)
Mammals, effect of exogenous estrogens on male (EMMENS). V, 233 (1947)
Metabolism of gonadotropic hormones in the organism (ZONDEK). III, 297 (1945)

—, intermediate, of the sex hormones (Pincus). I, 294 (1943)
Microbiology, growth factors in (Knight). III, 108 (1945)
Mouse, vitamin requirements (Morris). V, 174 (1947)
Neurophysiology, thiamine and peripheral (Muralt). V, 93 (1947)
Nutritional Deficiency, manifestations in infants (Clements). IV, 73 (1946)
——, manifestations of prenatal (Warkany). III, 73 (1945)
— states, appraisal (Jôliffe). I, 60 (1943)
Primates, nutritional requirements of, other than man (Day). II, 71 (1944)
Proteins, thyroactive iodinated (Reineke). IV, 207 (1946)
Protozoa, growth factors for (Hall). I, 249 (1943)
Pteroylglutamates, physiological effects in man (Darby). V, 119 (1947)
Pteroylglutamic acid, physiological effects in man (Darby). V, 119 (1947)
Reproduction, effect of B-vitamins on the endocrinological aspects of (Hertz). IV, 135 (1946)
Sex Hormones, intermediate metabolism (Pincus). I, 294 (1943)
Sterols, X-ray crystallography and structure of (Crowfoot). II, 409 (1944)
Sulfonamides and vitamin deficiencies (Daft). III, 49 (1945)
Testosterone, clinical use in the male (Heller). V, 392 (1947)
Thiamine and peripheral neurophysiology (Muralt). V, 93, (1947)
Thyroid and diabetes (Houssay). IV, 188 (1946)
Tissues, significance of vitamin content (Williams). I, 229 (1943)
Vitamin A, chemistry and physiology (Heilbron). II, 155 (1944)
——, photo-receptor function of, and carotenoids (Wald). I, 195 (1943)
——, synthesis of, and of related products (Milas). V, I (1947)
Vitamin B synthesis by intestinal bacteria (Najjar). III, 23 (1945)
Vitamin B-Complex, newer hematopoietic factors of (Pfiffner). IV, 1 (1946)
Vitamin E, physiological action of, and its homologues (Mason). II, 107 (1944)
Vitamin deficiency and sulfonamides (Daft). III, 49 (1945)
Vitamins and cancer (Burk). II, 306 (1944)
—, chemical and physiological relationship between, and amino acids (Mitchell). I, 157 (1943)
—, effect of B-, on the endocrinological aspects of reproduction (Hertz). IV, 135 (1946)
—, interrelation of (Moore). III, (1945)
—, physical methods for identification and assay of, and hormones (Loofbourow). I, 109 (1943)
—, physiological availability (Melnick). V, 40 (1947)
—, requirement of chicks (Bird). V, 163 (1947)
—, —— mouse (Morris). V, 174 (1947)
—, role of, in the anabolism of fats (McHenry). II, 1 (1944)
Women, use of androgens in (Carter). V, 315 (1947)
X-Ray Crystallography and sterol structure (Crowfoot). II, 409 (1944)